A Developmental Perspective on the Origin of Morphological Disparity in Domesticated Horses

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Dissertation

A Developmental Perspective on the Origin of Morphological Disparity in Domesticated Horses

The morphological disparity of domesticated animals provides a rich subject to investigate the developmental bases of shape variation and its limitations within a species. Additionally, it offers a worthwhile comparison with evolutionary patterns at the macroevolutionary scale. This PhD thesis aims to investigate the influence of domestication on life history variables and morphological disparity among horses. It has been claimed that in horses, morphological and life history changes from wild relatives have been less pronounced than in the case of other domesticated mammals, e.g. dogs or pigs. Empirical studies, however, including various breeds, are scarce.

Chapter 1 aims to investigate the variation in gestation length among different horse breeds and its relation to size. Differences in prenatal growth, e.g. expressed by differences in average gestation length, are potentially linked to size and shape differences among breeds. In horses, it has been claimed that the variation in gestation length is particularly high. Some of the environmental and genetic factors influencing gestation length are already known, while the influences of breed affiliation and size have rarely been studied. Results indicate an influence of domestication on the gestation length in domesticated horses by presenting differences in average gestation lengths among breeds. Size, however, is not linked to gestation length in horses, hinting towards differences in prenatal growth rates among breeds as the reason for size differences at birth.

Chapter 2 examines how domestication and strictly controlled breeding influence gestation length in various domesticated mammal species. As gestation length is an important life history variable for production management, e.g. in the milk industry, the variation of gestation length should be low in stricter controlled production animals such as cattle, pigs, sheep, and goats. It has further been hypothesized that the variation of gestation length in horses is higher than in other domesticated mammals. Comparison of gestation lengths from the literature for production and non-production animals showed that production animals have a decreased variation in their gestation length and that horses fall well within the range of non-production animals. With their higher variation, goats form an exception to the rule, probably based on their less constrained breeding standards.

Chapter 3 aims, for the first time, to assess, quantify, and evaluate the morphological differences and patterns of modularity in cranium, mandible, and teeth between domesticated horses and extant wild equids. Results show a larger shape variation of domesticated horses compared to wild equids in all tested features. The crania of domesticated horses group with the Przewalski's horses and tend to separate from zebras and donkeys in shape space, while mandible and teeth shapes are indistinguishable among groups. Furthermore, six cranial modules were confirmed with the lower values of integration in domesticated horses than in wild equids, being associated with higher disparity values across all cranial modules in the former.

In Chapter 4 it was investigated if novel skull shapes in horses represent a form of paedomorphism. Due do their considerable size range, horses are an excellent study subject to investigate patterns of size-related shape change. Using three-dimensional geometric morphometrics, allometric growth could be shown in ontogenetic series of horse skulls. The two investigated miniature breeds, Falabella and Shetland pony, are not miniature forms of regular-sized breeds, but exhibit paedomorphic features, such as enlarged orbits relative to cranial length

and larger crania compared to wither height.

The results of this PhD thesis show that domestication and artificial selection greatly increased the variation in the life history and skull shape of horses.

Die morphologische Variation von domestizierten Tieren bietet ein reiches Angebot, um die entwicklungsbiologischen Grundlagen und die Limitierungen von Formveränderungen innerhalb einer Art zu erforschen. Zusätzlich bietet es lohnenswerte Vergleichsmöglichkeiten mit evolutionsbiologischen Vorgängen auf makroevolutionärer Ebene. Diese Dissertation hat die Absicht, den Einfluss der Domestizierung auf den Lebenszyklus und die morphologische Variation in Pferden zu untersuchen. Es wird behauptet, dass die Veränderungen im Lebenszyklus und in der morphologischen Variation im Vergleich zum wilden Vorfahren bei Pferden geringer sind als bei anderen domestizierten Tieren, zum Beispiel Hunden oder Schweinen. Empirische Studien, die mehrere Rassen beinhalten, sind jedoch selten.

Kapitel 1 hat zum Ziel die Variation der Tragzeitlänge bei unterschiedlichen Pferderassen zu untersuchen und inwiefern diese mit der Körpergrösse zusammenhängt. Unterschiede im pränatalen Wachstum, die sich zum Beispiel durch Unterschiede in der durchschnittlichen Tragzeitlänge äussern, stehen vermutlich in Verbindung mit Grössen- und Formunterschieden zwischen den Rassen. Es wird behauptet, dass die Variation in der Tragzeitlänge bei Pferden besonders gross ist. Einige der ökologischen und genetischen Faktoren, die die Tragzeitlänge beeinflussen sind bereits bekannt, während der Einfluss von Rasse und Körpergrösse wenig untersucht wurde. Die Ergebnisse dieses Kapitels weisen auf einen Einfluss der Domestizierung auf die Tragzeitlänge bei domestizierten Pferden hin, da es signifikante Unterschiede in der durchschnittlichen Tragzeitlänge zwischen den Rassen gibt. Die Körpergrösse hat jedoch keinen Einfluss auf die Tragzeitlänge in Pferden, was demnach auf Unterschiede in der pränatalen Wachstumsrate als Grund für die unterschiedlichen Körpergrössen bei der Geburt hindeutet.

Kapitel 2 untersucht den Einfluss der Domestizierung und der streng kontrollierten Zucht auf die Tragzeitlänge bei verschiedenen domestizierten Arten. Da Tragzeitlänge ein wichtiger Faktor für die Industrie ist, wie zum Beispiel in der Milchproduktion, sollte die Variation der Tragzeitlänge bei strenger kontrollierten Produktionstieren, wie Rindern, Schweinen, Schafen oder Ziegen, geringer sein. Des Weiteren wurde die Hypothese geäussert, dass die Variation der Tragzeitlänge in Pferden höher sei als bei anderen domestizierten Arten. Ein Vergleich von Tragzeitlängen aus der Literatur für Produktionstiere und Nicht-Produktionstiere zeigt, dass Produktionstiere eine geringere Variation ihrer Tragzeitlänge haben und dass Pferde im normalen Bereich von Nicht-Produktionstieren liegen. Ziegen bilden mit ihrer hohen Variation eine Ausnahme, was vermutlich auf ihre weniger streng kontrollierte Zucht zurückzuführen ist.

Kapitel 3 hat die Absicht zum ersten Mal die morphologischen Unterschiede und die Muster der Modularität im Schädel, im Unterkiefer und in den Zähnen zwischen domestizierten Pferden und noch vorhandenen Wildequiden festzustellen, zu quantifizieren und zu evaluieren. Die Ergebnisse zeigen eine höhere Formvariation von domestizierten Pferden im Vergleich zu Wildequiden in allen getesteten Merkmalen. Die Schädel der domestizierten Pferde gruppieren sich mit den Przewalskipferden und tendieren dazu sich von den Zebras und Eseln abzutrennen, während sich die Gruppen anhand des Unterkiefers und der Zähne nicht unterscheiden lassen. Des Weiteren wurden sechs Module im Schädel bestätigt und die niedrigeren Werte für Integrität bei domestizierten Pferden als bei Wildequiden können mit einer höheren Disparität in Verbindung gebracht werden.

Kapitel 4 zielt darauf ab zu untersuchen ob neue Schädelformen bei Pferden eine Form von

Pädomorphismus darstellen können. Aufgrund ihrer beträchtlichen Grössenvariation bieten Pferde ein ideales Forschungsobjekt um Muster in grössenverwandten Formunterschieden zu untersuchen. Mit Hilfe von dreidimensionalen geometrisch-morphometrischen Analysen konnte allometrisches Wachstum in einer ontogenetischen Serie von Pferdeschädeln nachgewiesen werden. Die beiden untersuchten Miniaturpferderassen, Falabella und Shetland Pony, sind keine Miniaturformen von normalgrossen Pferderassen, sondern zeigen pädomorphe Merkmale, wie zum Beispiel vergrösserte Augenhöhlen in Relation zur Schädellänge und längere Schädel im Vergleich zum Stockmass.

Die Ergebnisse dieser Dissertation zeigen, dass die Domestizierung und die künstliche Selektion die Variation des Lebenszyklus und der Schädelform von Pferden stark beeinflusst hat.

Introduction

Humankind has experienced three major surges in cultural evolution, which drastically altered human lifestyle: the beginning of tool use, the Neolithic revolution, and industrialization (Clutton-Brock 1999). The Neolithic revolution, the transition from hunting and gathering to agriculture and with it the rise of civilization, has played a major role in reshaping the organization of human societies (Weisdorf 2005; Vigne 2011; Zeder 2012; Larson and Fuller 2014). From all the animals that have been domesticated during and since the Neolithic revolution, arguably none influenced human society as much as the horse (*Equus ferus caballus*). It revolutionized transport and provided horse-riding societies great advantages over others, until the invention of the combustion engine. Horses could not only travel further and faster, but other work such as herding or farming was facilitated (Anthony 2010). Although the importance of the horse changed from work to leisure after industrialization, we still experience the effects of this domestication today. Investigating when, where, and how domestication, not only of the horse but in general, took place is crucial to understand the roots of human societies (Larson and Fuller 2014). Although often displayed as a one-time rapid event caused by human intention, the process of domestication is long and complex with many participants and factors involved (Smith 1998; Larson and Fuller 2014). In the following paragraphs of this introduction, I would like the reader to converge to the topic of domestication in general and the domestication of horses in particular, including a discussion of accompanying changes and underlying evolutionary mechanisms.

What is domestication?

Domestication and taming are two processes describing human-animal interactions. A distinction between the two is sometimes challenging, as opinions in the literature vary substantially. Some researchers interpret domestication as a goal-oriented process where humans deliberately and with intention gain control over an animal's life (Reed 1959; Hale 1969; Clutton-Brock 1981, 1994). This definition can also be used for taming, which describes the process of extracting animals from the wild and habituating them to the human environment without producing tame offspring through breeding, e.g., elephants in Asia (Diamond 2002). A second group of authors has defined domestication more as a mutualistic partnership in which both partners benefit (Budiansky 1992; Morey 1992). Viewing domestication from the animal's perspective, it can also be said that it is the colonization of and adaptation to an anthropogenically created niche (Herre and Röhrs 1973; Zeder 2012; Larson and Fuller 2014). I tend to agree with the more recent definition of domestication as a result of co-evolutionary mutualism that developed in the context of active niche construction by both humans and the domesticated species (Zeder 2015; Smith 2016; Zeder 2016). Generally it needs to be pointed out that domestication is a gradual process with continuous transitions from one state to another and thus an exact definition is neither possible nor desirable due to the lack of a universal threshold (Roots 2007; Driscoll *et al.* 2009; Vigne 2011; Zeder 2012).

Prerequisites and the pathways of domestication

From all of today's large herbivore mammal species, only around 10% have been successfully domesticated (Diamond 1998). Some researcher state that in order to become domesticated, a species needs to show certain prerequisites: (1) they need a certain type of social group structure (hierarchical, affiliation of males) (2) they need to have a promiscuous mating system with dominant males (3) they need social bonds with their offspring (4) they should be a generalist feeder and easy to tend, and (5) show a liking for humans (short flight distance, comfort loving) (Galton 1865; Price 1984; Clutton-Brock 1999; Roots 2007; Zeder 2012). In other words, it can be said that in order to be domesticated animals cannot exhibit slow growth and reproduction cycles, nasty disposition, reluctance to breed in captivity, lack of hierarchies, and a high level of flight response (Diamond 2002). Not all the domesticated species, however, have all the prerequisites or lack all the obstacles.

Depending on the prerequisites of the species, the importance of the resource and the level of human investment (Zeder 2012), the pathway of how a species became domesticated varies. All domestication processes which occurred until today can be classified into one of three pathways: the commensal pathway, the prey pathway, or the directed pathway (Zeder 2012; Larson and Fuller 2014). They vary on how they are initiated, the direction they take and the timeframe they are travelled in. The commensal pathway, most likely the first to have occurred, describes the domestication of animals which primarily fed on waste or leftovers around human settlements or fed on prey which did that. The animal species then established a commensal bond with humans which led the way to domestication. Examples are dogs, cats, rodents, and possibly pigs. The prey pathway was traveled by most major livestock species (e.g., cattle, sheep, goats), all being former prey species commonly hunted by humans. Humans, most likely experienced with hunting strategies, pursue the goal to increase prey availability. Hunting developed into game-management which then developed into herd-management. In the beginning of this pathway, a frequent introgression between wild and captive animals and restocking the herd from the wild is likely (Larson and Fuller, 2014). The third pathway, the directed, is a more human intended process. For this pathway to work, humans needed the knowledge of how to domesticate a species from one of the two previous pathways. The species domesticated through this pathway are likely not to fulfill all prerequisites for domestication. Horses are the prime example for directed domestication.

Domestication of horses

Prior to their domestication, horses were at the verge of extinction (McFadden 1992). During the late Pleistocene large herds of wild horses roamed the steppes of Eurasia and North America. At the end of the last glacial period, their natural habitat, the steppe, was replaced by dense forest in many places of the world. Horses became extinct in North America probably due to changing climatic conditions and extensive hunting (McFadden 1992). The last existing large herds in Europe and Asia migrated into the open grasslands of Eurasia, where they became the most common large mammals (together with *Equus hemionus* and *Equus hydruntinus*, Anthony 2010). The Eurasian herds suffered

massive losses and only very few remains can be found between the last ice age and the beginning of their domestication (Bokonyi 1974).

When horses were first domesticated during the Neolithic, a long lasting relationship of humans and horses must have already existed (Anthony 2010). It is likely that the first humans, who domesticated horses, were familiar with wild horses. Thus, they must have lived in places where they were in frequent contact with these animals and probably hunting them. The first signs of horse domestication have been confined to two possible sites in the Eurasian steppe: Dereivka and Botai (Levine 1999 and references therein), where nomads concentrated strongly on hunting wild horses. The primary reason for horse domestication was most likely the search for an additional food source (Clutton-Brock 1981; Olsen 2006; Vilá *et al.* 2006). Although the nomads had already domesticated other livestock species such as sheep and cattle, they might have been in need for additional food sources due to harsher winter (Anthony 2010). However, the reason for the horses´ outstanding importance to human societies is not their edibility, but their transformation of transportation. Whether horses were first loaded with goods, put in front of a carriage, or ridden is difficult to establish. Nevertheless, they transformed and dominated the mode of transportation drastically until they were replaced in most parts of the world by the invention of the combustion engine in the 20th century. The first civilizations to domesticate horses gained great advantages over others. They were able to travel faster and further than anyone and history shows that they conquered large parts of the Old World, e.g., Genghis Khan (Turnbull 2014). Along with them came the knowledge on how to capture, tame and rear wild horses, as well as language, culture, and goods, which had a profound impact on the course of civilization (Vilà *et al.* 2001).

The exact timing of the first domestication in horses is difficult to determine. Different archaeological relics such as the presence of drawings, bits, corrals, or horsemilk residue are currently used to determine the presence of domesticated horses (Outram *et al.* 2009). Hybridization and introgression are widespread in early domestication, which hinders the development of strong morphological differences at the onset of domestication. It further increases genomic affinity in modern breeds to wild populations that were not part of the original domestication process, which complicates evaluating the results of genomic analyses (Larson and Fuller 2014). Yet, those analyses based on modern horses hint towards a strong sex bias during domestication with the maternal bloodline showing high diversity and the paternal bloodline showing high homogeneity (Vilà *et al.* 2001; Jansen *et al.* 2002; Lindgren *et al.* 2004; Anthony 2010). This structure is consistent with modern breeding practices (Gordon 2017) and based on a trading bias towards mares. Mares are easier to handle than stallions, which promotes a breeding system where one stallion covers many mares. This breeding practice significantly reduces patrilines (Cunningham *et al.* 2001).

Underlying patterns of and changes in domestication

The morphological and physiological changes in a species are mainly secondary artifacts accompanying the selection for tameness and the relaxation of natural selection pressures (Zeder 2006a, b, 2012). This coupling was demonstrated by a long

term experiment on silver foxes led by Dmitry Belyaev, who showed that those individuals which were only selected for tameness showed very distinct traits (Trut 1999; Trut *et al.* 2009). Many of these traits were already noticed by Darwin (1859), occur in various domesticated species but not in all, as they are not universal (Vigne 2011; Sánchez-Villagra *et al.* 2016). The full set of these traits includes: a reduction in size, change of fur color and type, a curly tail, floppy and/or larger ears, decrease in brain size, changes in craniofacial morphology including a shorter rostrum, smaller teeth, more frequent and nonseasonal estrus cycles, prolongations of juvenile behavior, and changes in hormone levels (Clutton-Brock 1981; Vigne 2011, see references in Table 1 Wilkins *et al.* 2014). This set of traits, the 'domestication syndrome' (Wilkins *et al.* 2014, is not meant as a clinical term but as a complex of traits. The fact that the selection for tameness led to the domestication syndrome is hypothesized to be based directly or indirectly on mild neural crest deficiencies during embryonic development (Wilkins *et al.* 2014). While deficiency of neural crest cells is hypothesized to cause most of the traits coupled with domestication, compelling evidence for this hypothesis is still lacking (Sánchez-Villagra *et al.* 2016).

Humans could not have foreseen the morphological and physiological change that would come alongside a tamer behavior but they were able to evaluate the changes and therefore continue or modify them in later stages (Zeder 2012). At some point during domestication humans started to "improve" the domesticates by artificially selecting animals and traits for economic, cultural, or aesthetic reasons (Clutton-Brock 1981). This intense selective breeding is the reason for very drastic morphological and physiological changes that we can see today in some species (e.g. dogs), however, the changes are always canalized by biological constraints (Clutton-Brock 1981; Driscoll *et al.* 2009). Furthermore, it has been shown that some of the traits are reversed by feralisation, e.g., coat color, while others remain, e.g., reduced brain size (Kruska 1988). Research on this topic is complicated by the fact that most of the morphological changes that we see today in our domesticated species are single, decoupled features, which were modified by intense selective breeding and are thus not part of the initial domestication syndrome.

Many of the changes associated with domestication are further hypothesized to link to changes in developmental rate or timing (Clutton-Brock 1981). Shape differences in dog skulls, for example, have long been hypothesized to be generated by the retention of juvenile characters from their ancestors (Wayne 1986; Morey 1992; Goodwin *et al.* 1997). However recently it has been shown that, a combination of paedo-,pera-, and neomorphism patterns characterize dog skull growth compared to that of wolves (Geiger *et al.* 2017).

Why do research on domestication?

Domestication can be seen as an experiment in evolution, one that leads to morphologically different types in rapid time which otherwise can only be found on a geological timescale (Herre and Röhrs 1973). Understanding the evolutionary mechanisms underlying domestication might provide valuable insights into evolutionary processes and can be used as a model system for phenomena such as island evolution (Sánchez-Villagra *et al.* 2016). The study of domestication further provides us with valuable information about rate and

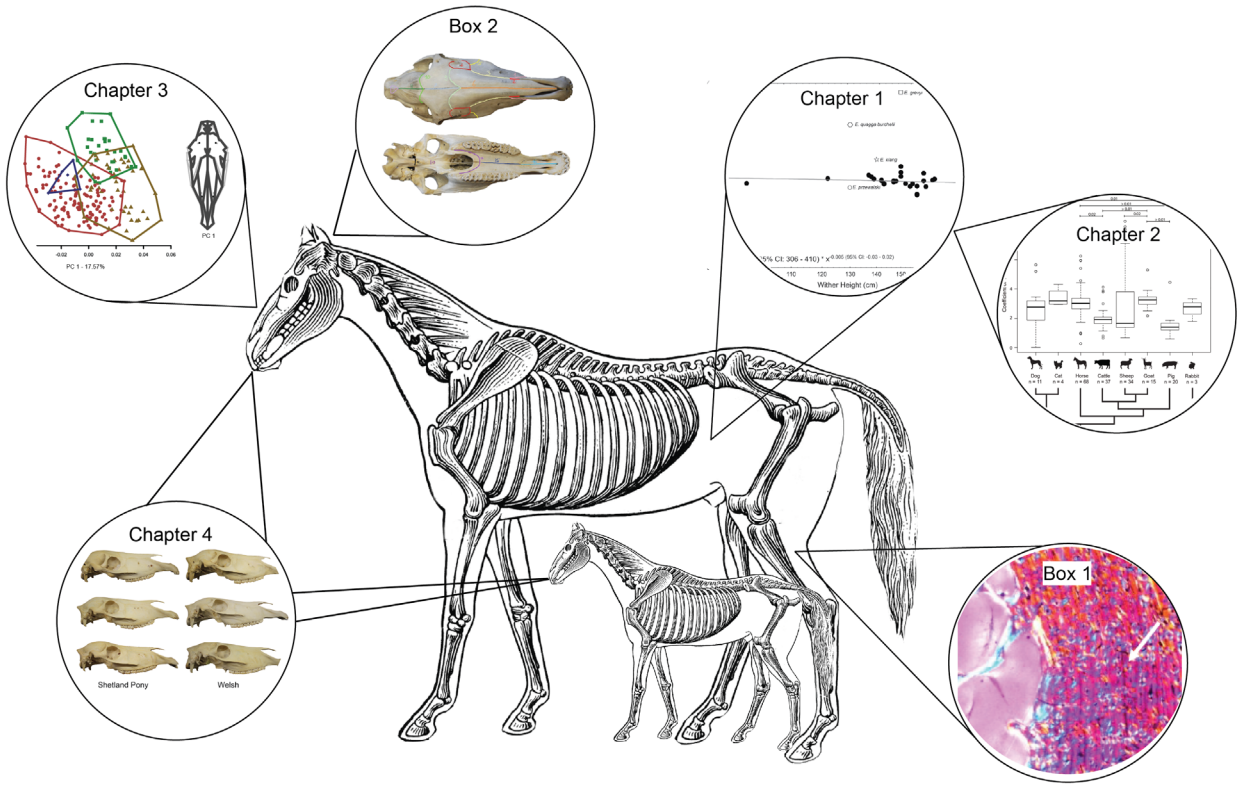

Figure 1: Illustration of the different chapters of this PhD thesis. Chapters 1 and 2 are concerned with the variation of gestation length in horses (Chapter 1) and various domesticated species (Chapter 2). Chapters 3 and 4 analyse the morphological variation of the skull between domesticated horses and wild equids (Chapter 3) and within ontogenetic series of different sized horse breeds (Chapter 4). The boxes represent small preliminary investigations on growth rate estimation using bone histology (Box 1) and patterns of suture closure (Box 2), which will be presented in the conclusion of this thesis. Artwork is modified after Davison 1906.

extent of phenotypic changes and possibly its limitations.

Research on archaeological remains such as pottery or art, as well as the comparison of skeletal remains, has provided valuable insights to the area of domestication. However, due to the limitations of these studies, their results are often vague. The advances in technologies are accompanied by new methods (e.g., genomics, geometric morphometrics, and CT-scanning) which might be able to answer some of the remaining questions regarding origin and evolutionary mechanisms involved (Larson and Fuller 2014; Evin *et al.* 2016; Schweizer *et al.* 2017).

Aims

On a daily basis, we can observe examples of morphological changes that have resulted from the domestication of a wild ancestor species. In some species, e.g., dogs or pigs, these morphological changes are extensive and obvious. In horses, however, morphological changes are less pronounced and the wild ancestor is extinct, complicating a direct comparison. The developmental bases behind the morphological changes and the resulting differences in breed diversity remain largely uninvestigated. The main aim of my PhD thesis is, therefore, to investigate the influence of domestication

on life history variables and morphological disparity among horses (Figure 1). To date, detailed studies on differences in life history traits or morphology related to domestication in horses are scarce or limited to a single breed. It has been shown that fewer traits are influenced in horses by domestication than in many other domesticates, and most of all as in dogs, which are a prime research example for domestication (Wilkins *et al.* 2014 and references therein). To understand the evolutionary mechanisms that work alongside domestication it is indispensable to first assess, quantify, and evaluate the changes caused by domestication and selective breeding in as many species as possible, and second compare similarities and differences among species.

This PhD thesis adds valuable data to the records of horse domestication from a developmental point of view. I investigate differences in gestation length among horse breeds (Chapter 1), differences in the variation of gestation length in various domesticated species (Chapter 2), morphological variation within the equid clade (Chapter 3), and a possible origin of disparity in domesticated horses (Chapter 4).

Chapter 1 of my thesis investigates whether domestication has influenced gestation length in horses. The latter has been claimed to be very variable (Aoki *et al.* 2013), however, studies including various breeds, and thus investigating a possible influence of domestication, are rare. In particular, I tested if gestation length is a flexible variable among breeds and if it is connected to size in horses.

During the investigation of gestation length variation in horses, we came across references stating that the gestation length among horses is larger than in any other domesticated species (Bos and Van der Mey 1980; Aoki *et al.* 2013). In contrast to that, we hypothesized that the level of control over breeding should have an influence on variation of gestation length and therefore more strictly controlled production animals should have less variation in their gestation length than non-production animals, which we tested using available literature data. We considered phylogenetic relationships for this study, since it has been shown that changes in life history traits associated with domestication might be affected by phylogeny (Francis 2015; Sánchez-Villagra *et al.* 2016). This study is Chapter 2.

Chapter 3 assesses, quantifies, and compares, for the first time, morphological differences in cranium, mandible, and teeth of domesticated horses and extant wild equids. This assessment has been previously done in dogs showing that morphological variation in domesticated dogs exceeded that of wild species and is comparable to disparity found in Carnivora (Drake and Klingenberg 2010). Darwin (1868) hypothesized a similar situation for horses, suggesting that the intraspecific variation is larger than the interspecific disparity of equids in general. We tested Darwin's hypothesis and further investigated whether the patterning of disparity in horse crania reflects a modular pattern. In particular, we analyzed different hypotheses for modularity (Cheverud 1982; Radinsky 1984; Goswami 2006) and quantified the magnitude of modularity and integration between domesticated horses and wild equids.

Chapter 4 focusses on the intraspecific cranial disparity in domesticated horses in an ontogenetic context. Particular interest was given to two miniature breeds, Falabella and Shetland pony, where shape novelties were investigated in the light of heterochrony and

allometry. We used geometric morphometric methods, linear measurements, and multivariate analyses to study postnatal growth of crania including a diversity of breeds. Since morphological changes can occur in a modular fashion in the cranium (Gilmour *et al.* 1993), we investigated if some individual cranial modules show more ontogenetic differences than others across all breeds.

Appended to this thesis is an additional manuscript (co-authorship) comparing postnatal skull growth in 13 domesticated species and their wild ancestors. The study investigates the similarities and differences in growth patterns produced by domestication.

Thesis outline

In this cumulative thesis the chapters are presented as a fully-formatted article as published in the respective journal (*Chapters 1 and 2, and Appendix*) or in manuscript form (*Chapters 3 and 4*). Authors and publication details are provided at the beginning of each chapter. Author/co-author contributions for all chapters are provided at the end, prior to the CV. Supplementary materials are attached at the end of each chapter with the raw data provided upon request.

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Chapter I

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Original investigation

Gestation length variation in domesticated horses and its relation to breed and body size diversity

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ABSTRACT

The domestication process and selective breeding reportedly alter some life history variables. In horses, it has been claimed that gestation length is particularly variable. Some of the factors influencing gestation length are already known and can be grouped into environmental and genetic factors, but the effects of breed and body size have rarely been evaluated. In this study we tested the influence of breed and body size on gestation length for 25 horse breeds from Central Europe. The mean gestation length for all breeds was 342.3 ± 10.2 days and we found significant differences among breeds with a variation of up to 11 days. Body size did not show a significant correlation with gestation length. Our data suggest that breed affiliation explains part of the large variability of gestation length in horses.

Introduction

The process of domestication can generate changes in animals' life history, including reproductive cycles (Geiger et al., 2016; Herre and Röhrs, 1990). Horses, for example, have a large variation in their gestation length and some researchers suggest that it is even larger than in their wild relatives (Aoki et al., 2013). Full term gestation lengths ranging between 294 and 419 days with viable foals have been described (Rossdale, 1976; West, 1994) but periods considered "normal" range from 300 to 380 days (Aoki et al., 2013; Pérez et al., 2003).

Gestation length is much affected by diverse environmental and individual variables (Meliani et al., 2011). A significantly longer gestation length in mares bred at the beginning of breeding season compared to mares bred at the end of breeding season has been confirmed by various studies (Cilek, 2009; Davies Morel et al., 2002; Dicken et al., 2012; Langlois and Blouin, 2012; Meliani et al., 2011; Pérez et al., 2003; Rezac et al., 2013; Satué et al., 2011; Sevinga et al., 2004; Valera et al., 2006). As it has been found in other species with seasonal activity, e.g. in Przewalski's horses (Bronson and Heideman, 1994; Chen et al., 2008), mares show this foaling accumulation in spring to ensure optimal conditions for the offspring's survival.

Differences in gestation length are also related to the sex of the fetus. It is generally accepted that gestation length has a gender bias in horses and is about 1–2 days longer if the mare carries a colt (Aoki et al., 2013; Bene et al., 2014; Cilek, 2009; Heidler et al., 2004; Hintz et al., 1992; Langlois and Blouin, 2012; Marteniuk et al., 1998; Pérez et al., 2003; Sevinga et al., 2004; Staffe, 1935; Taveira and da Mota, 2007; Uppenborn, 1933; Valera et al., 2006; Van Rijssen et al., 2010). This is hypothesized to be caused by testosterone or chromosome linked effects (Cilek, 2009), differences in the interaction with endocrine control of parturition (Jainudeen and Hafez, 2000), or a more developed allontochorion in colts (Wilsher and Allen, 2003). In contrast to the high number of publications on this subject, two studies could not find differences between male and female foals (El-Wishy et al., 1990; Valente et al., 2006).

Several studies discuss the influence of additional factors such as climate (Mauch, 1937), stud farm where the mares are located during the gestation period (Aoki et al., 2013; Davies Morel et al., 2002; Langlois and Blouin, 2012; Van Rijssen et al., 2010), feeding management (Thorson et al., 2010; Uppenborn, 1933), type of insemination (Bene et al., 2014), inbreeding (Langlois and Blouin, 2012), length of last gestation period (Aoki et al., 2013), interval from ovulation to mating (Davies Morel et al., 2002), unspecified characteristics of the individual mare (Giger et al., 1996; Uppenborn, 1933; Valera et al., 2006; Van Rijssen et al., 2010), reproductive status of the mare (Van Rijssen et al., 2010), parity of the mare (Aoki et al., 2013; El-Wishy et al., 1990; Pool-Anderson et al., 1994; Sanchez, 1998; Staffe, 1935; Valente et al., 2006; Valera et al., 2006; Winter et al., 2007), age of the mare (Aoki et al., 2013;

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Bene et al., 2014; Bos and Van der Mey, 1980; Cilek, 2009; Davies Morel et al., 2002; Demirci, 1988; Guay et al., 2002; Heidler et al., 2004; Hintz et al., 1992; Kurtz Filho et al., 1997; Langlois and Blouin, 2012; Mauch, 1937; Sevinga et al., 2004; Valera et al., 2006; Winter et al., 2007), unspecified characteristics of the individual stallion (Bene et al., 2014; Mauch, 1937; Van Rijssen et al., 2010), age of the stallion (Davies Morel et al., 2002), artificial light (Caldas et al., 1994; Palmer and Driancourt, 1983), or year of breeding (Cilek, 2009; Langlois and Blouin, 2012; Valera et al., 2006).

Two additional factors are worth considering when examining variation on gestation length: breed and body size. Many of the studies related to gestation length were conducted with a single breed (Caldas et al., 1994; Cilek, 2009; Heidler et al., 2004; Hintz et al., 1992; Howell and Rollins, 1951; Pérez et al., 2003; Rollins and Howell, 1951; Taveira and da Mota, 2007; Van Rijssen et al., 2010; Winter et al., 2007) and results of those including different breeds are contradictory (Bene et al., 2014; Bos and Van der Mey, 1980; Langlois and Blouin, 2012; Roberts, 1986; Valera et al., 2006). One study showed significant variation of up to six days in the average gestation length among breeds (Bos and Van der Mey, 1980). One review reported differences of up to 13 days among the mean gestation length of different breeds (Satué et al., 2011). The authors argued, however, that the differences among previous reports might not only be caused by breed but also by differences in the way the gestation period was calculated, or differences in climate or photoperiod. On the other hand, no significant differences in gestation length among Hungarian horse breeds were reported by Bene et al. (2014).

Thus, previous studies provide a good overview of factors influencing gestation length regarding individual horse breeds; however, the influence of breed itself and body size remains unclear. In this study, we aim to investigate how those two factors influence the gestation length of the horse. Since research on gestation length of various horse breeds showed significant differences among breeds (Bos and Van der Mey, 1980; Langlois and Blouin, 2012; Valera et al., 2006), we firstly hypothesized that gestation length is a flexible life history variable in horses which does vary among breeds. Secondly, previous research on other domesticated species shows no significant correlation between size and gestation length in different sized breeds (reviewed in Clauss et al., 2014). Thus, we hypothesize that body size does not have a significant influence on gestation length.

Material and methods

In the present study, we used a total of 30,792 gestation lengths (15,599 female and 15,193 male newborns) from 25 horse breeds (Table 1). All gestation lengths were recorded by studbook societies in Germany and Switzerland (Rheinisches Pferdestammbuch e.V., Schweizer Freiberger Verband) which collected their data in Central Europe. Selected gestation lengths were pregnancies which resulted in one single viable foal during the breeding periods from 2000 to 2015. The duration of a gestation length was determined by the time interval between the last day of mounting/insemination and the day of parturition. Information on the age or parity of the mares was not consistently available in the dataset we collated. We restricted our dataset to gestation lengths ranging from 300 to 380 days due to previous studies referring to a normal gestation length in this range (320–360 in Laing and Leech (1975); Rossdale (1976), 300–400 in Pérez et al. (2003), 300–380 in Aoki et al. (2013)). In addition, only breeds with a sample size of at least ten individuals were used for the analyses. To visualize the position of wild equids in the allometric linear regression, we added average gestation lengths of four wild equid species from the literature to the figure but did not include them in the statistical analyses (Fig. 2).

Previous studies showed that foal gender and month of insemination have a significant influence on the gestation length of horses (see Introduction). Since we aimed to investigate the influence of the breed on gestation length, we calculated a model where the gestation length was linearly adjusted by the influence of foal gender and month of insemination (Linear model: Adjusted gestation length = $a + b^{*}sex + c_i{}^{*}month + \varepsilon$; a, b, $c_i \in R$; $\varepsilon \sim N(0,1)$; $i \in \{1,\ldots,12\}$). This adjustment is important to isolate the part of the gestation length which is explained by the breed only. We adjusted every recorded gestation length accordingly; in other words, all data presented in this study were adjusted in this manner. In 36 cases, the adjustment led to gestation lengths above the 380 days, which we still included in the following analyses. Differences in gestation lengths among breeds were evaluated using a parametric analysis of variance (ANOVA) followed by a post-hoc Tukey test and a Kolmogorow-Smirnow test of the residuals. To investigate the influence of body size on gestation length in our second analysis, we conducted an allometric linear regression. As a proxy for body size we used mean wither height of each breed according to breeding standards from the literature, since no individual height or weight was indicated in the available data. In contrast to wither height breed standards, no standard body mass data exist for all the breeds. We correlated the mean wither height to the breed's average gestation length using log-transformed data. The resulting coefficient in the allometric equation is stated, including its 95% confidence intervals in brackets. All statistical analyses were performed using Statistica (Version 12, StatSoft Inc., Tulsa, USA) and the significance for all tests was set at $\alpha = 0.05$. Results are displayed as means ± standard deviation.

Results

The gestation length mean for the 25 breeds was 342.3 ± 10.2 days, with a range between individual animals of 301–388 days (Table 1). The ANOVA resulted in significant differences between the 25 breeds ($p < 0.0001$, $F = 13$, for sample size see Table 1) with a maximum mean gestation length of 351 days in Welsh Cobs and a minimum mean gestation length of 340 days in Friesians leading to a variation of 11 days among all means (Fig. 1). The post-hoc Tukey tests resulted in 9 out of 300 comparisons which were significant, mainly involving comparisons of Welsh Cobs and Rhenish Warmbloods with other breeds.

Our second analysis, the allometric linear regression, showed no correlation between mean wither height and mean gestation length in our sample (Fig. 2). The allometric exponent was low with mean wither height scaling to gestation length$^{-0.005}$ (95% CI: −0.03–0.02, $r^2 = 0.007$, $p = 0.67$).

Discussion

Breed affiliation is an important factor influencing variation in gestation length in horses, whereas the effect of body size is not significant. However, parturition time in horses is difficult to predict due to its high variability and unclear indicating signs.

Not all factors which potentially influence gestation length have been examined so far. In this study, we tested the influence of breed and body size on gestation length among 25 breeds.

Differences in calculation of gestation length might introduce some error when comparing studies. Gestation length is often calculated, like in our study, as the period between last mating (or insemination) and parturition. This period, however, is not equivalent to the true gestation length: the period between ovulation and parturition. Galisteo and Perez-Marin (2010) reported that in

Table 1
Gestation length in days with standard deviation sorted by breed; additional information on standard deviation, range, and n = sample size; empty cells equals not available.

Authors	Mean	±SD	Range	n_{total}	n_{female}	n_{male}	Breed or Species
This study	345.9	9.2		30	19	11	Aegidienberger
This study	340.7	18.5		10	6	4	American Saddlebred
Valera et al. (2006)	336.8	11.1	290–361	532	261	271	Andalusian
This study	342	8.8		48	25	23	Arab
Cilek (2009)	334.3	10.3	332–335	2189	1066	1123	Arab
Howell and Rollins (1951)	336.4			186			Arab
Vesović (1953)[1]	333.7						Arab
Pozo Lora (1954)[4]	343	11.4					Arab
Demirci (1988)[4]			314–361				Arab
El-Wishy et al. (1990)	332.1	3.3	300–371	1570			Arab
Valera et al. (2006)	340.3	9.7	306–360	234	129	105	Arab
Meliani et al. (2011)	332.95	8.6		1262	635	627	Arab
Ali et al. (2014)	335.5	10.2	320–360				Arab
Valente et al. (2006)	330.42	9.9		147			Arab
Mauch (1937)	338.25	10.2		521	261	260	Arab
This study	340.6	5.9		67	32	35	Barb
Bettini (1955)[1]	333.8						Belgian
Becze (1958)[1]	336.5						Belgian
This study	344	9.3		60	28	32	Black Forest Coldblood
Hrasnica (1944)[1]	339.1	0.9					Bosnian Pony
Satué (2004)[4]	332.4		297–358	44			Carthusian
Pérez et al. (1997)[4]			322–359	38			Carthusian Spanishbred
Pérez et al. (2003)	338.95	9.6	319–359	364			Carthusian Spanishbred
This study	345.7	9.8		27	14	13	Connemara Pony
Winter et al. (2007)	335.6	10.5	312–364	70			Criollo
Rezac et al. (2013)	339.2	11.3	305–392	321	165	156	Czech Warmblood
Bos and van der Mey (1980)	343.3			2002	1242	760	Draught Horse
This study	343.5	10.2		127	63	64	Fjord
This study	347.6	12.2		40	23	17	Frederiksborg
This study	341.9	10		23700	12060	11640	Freiberger
Giger et al. (1996)[4]	336.5		307–361	193			Freiberger
This study	340.5	11.3		56	31	25	Friesian
Sevinga et al. (2004)	331.6			495			Friesian
Bos and van der Mey (1980)	337.7			426	236	190	Friesian
Bene et al. (2014)	334.3	12.9		47			Furioso–North Star
This study	342	10.7		1080	549	531	German Riding Pony
Mauch (1937)	336.29	11.2		424	224	200	Gidran
This study	341.7	7.5		264	130	134	Haflinger
Matassino (1962)[4]	337.8	13					Haflinger
Bos and van der Mey (1980)	341.3			1034	593	441	Haflinger
This study	343.5	7.9		26	16	10	Haflinger (Noble Blood)
Bene et al. (2014)	335.2	14.9		122			Hungarian Cold Blooded
Bene et al. (2014)	333.6	19.8		146			Hungarian Sport Horse
This study	344.5	9.9		122	64	58	Islandic Horse
Bene et al. (2014)	336.2	14.8		123			Kisberi
Ilancic (1958)[1]	333.5						Lipizzaner
Heidler et al. (2004)	334.3	7.3		46	24	22	Lipizzaner
Bene et al. (2014)	334	9.8		60			Lipizzaner
Mauch (1937)	333.88	9.3		252	130	122	Lipizzaner
This study	348.1	13		15	8	7	Mangalarga Marchador
This study	342	8.7		28	13	15	Missouri Fox Trotter
Hura et al. (1997)[4]	332.4	6.9		298			Nonius
Bene et al. (2014)	335.1	15.3		146			Nonius
Mauch (1937)	335.92	10.4		432	207	225	Nonius
This study	343.5	10.5		15	7	8	Noriker
This study	343.4	10.2		39	14	25	Paso Peruano
Bettini (1955)[1]	342.2						Percheron
Aoki et al. (2013)	334.9	8.3	313–352	209	103	106	Percheron Mix
Zwolinski(1964)[4]			299–375				Polish
Pool-Anderson et al. (1994)	343/333			12			Quarter Horse
Guay et al. (2002)			339–344	12			Quarter Horse
Duggan et al. (2008)[4]			317–375	26			Quarter Horse
This study	344.5	10.6		4647	2305	2342	Rhenisch Warmblood
This study	343.9	11.7		122	59	63	Rhenish German Coldblood
Salerno and Montemurro (1966)	340.9	8.7					Salernitian
Bene et al. (2014)	333.3	12.7		36			Shagya
Walton and Hammod (1938)	333.3		323–343	3			Shetland
Bos and van der Mey (1980)	337.2			2327	1520	807	Shetland
First and Alm (1977)	334.4	3.17	322–344	7			Shetland
This study	342.1	14.5		168	74	94	Shetland
Pozo Lora (1954)[4]	343.6	12.2					Spanishbred
Blesa et al. (1999)[4]			322–346				Spanishbred
Pundir (2004)	333		308–365	36			Spiti

Table 1 (*Continued*)

Authors	Mean	±SD	Range	n_{total}	n_{female}	n_{male}	Breed or Species
Villani and Romano (2008)	337	7		350			Standardbred
Marteniuk et al. (1998)[4]			302–383	296			Standardbred
Dicken et al. (2012)	349	9.3	303–384	614[5]	313	295	Standardbred
Evans (2010)	349		312–393	1109	553	556	Standardbred
Tischner (1985)	325			2			Tarpan
Detkens (1963)[1]	338.8						Thoroughbred
Hintz et al. (1992)[4]			319–364				Thoroughbred
Allen et al. (2002)[4]			325–339	14			Thoroughbred
Duggan et al. (2008)[4]			322–366	18			Thoroughbred
Kurtz Filho et al. (1997)	334		315–360	390			Thoroughbred
Davies Morel et al. (2002)	344.1	0.5	315–388	433	238	195	Thoroughbred
Sanchez (1998)[2]	341.3	10.1	306–381	5472			Thoroughbred
Taveira and da Mota (2007)	337.83	9.5	302–396	25477	12927	12550	Thoroughbred
Elliott et al. (2009)			321–360	348	178	170	Thoroughbred
Van Rijssen et al. (2010)	352	10	309–398	627			Thoroughbred
This study	342.8	10.9		26	18	8	Tinker
This study	351.3	14.8		23	12	11	Welsh Cob
This study	344.7	8.6		29	19	10	Welsh Mountain Pony
This study	346.5	12.6		23	10	13	Welsh Pony
Pagan et al. (2009)			377–404				*Equus africanus somalicus*
Monfort et al. (1991)	340.2	6.8	331–352	6			*Equus ferus przewalskii*
King (1965)	399						*Equus grevyi*
Read et al. (1988)[3]	390						*Equus grevyi*
Asa et al. (2001)	407.3		391–425	3			*Equus grevyi*
King (1965)	371						*Equus quagga boehmi*
Wackernagel (1965)	371.2		361–390	28			*Equus quagga boehmi*
Brown (1936)	347		340–354	2			*Equus quagga burchellii*
Klingel (1969)[3]	381.5		378–385				*Equus quagga burchellii*
Smuts (1976)	396			1			*Equus quagga burchellii*
Joubert (1974)[3]	362						*Equus zebra hartmannae*
Penzhorn (1985)	359						*Equus zebra zebra*

[1] Cited in Salerno and Montemurro (1966).
[2] Cited in Perez et al. (2003).
[3] Cited Nuñez et al. (2011).
[4] Cited in Satué et al. (2011).
[5] Sex recorded for 608 individuals.

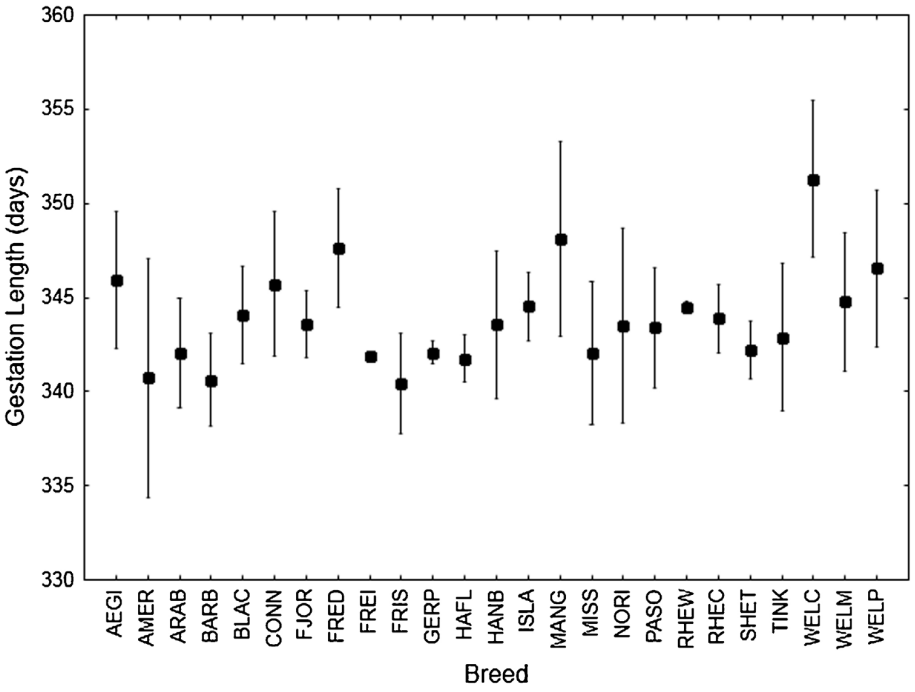

Fig. 1. Results of the ANOVA for all 25 horse breeds; data on gestation length are adjusted for foal sex and season (see Material and Methods); black squares denote the mean; vertical bars denote the 0.95 confidence intervals; AEGI (Aegidienberger), AMER (American Saddlebred), ARAB (Arab), BARB (Barb), BLAC (Black Forest Coldblood), CONN (Connemara Pony), FJOR (Fjord), FRED (Frederiksborg), FREI (Freiberger), FRIS (Friesian), GERP (German Riding Pony), HAFL (Haflinger), HANB (Haflinger Noble Blood), ISLA (Icelandic Horse), MANG (Mangalarga Marchador), MISS (Missouri Fox Trotter), NORI (Noriker), PASO (Paso Peruano), RHEW (Rhenish Warmblood), RHEC (Rhenish Coldblood), SHET (Shetland Pony), TINK (Tinker), WELC (Welsh Cob), WELM (Welsh Mountain Pony), WELP (Welsh Pony).

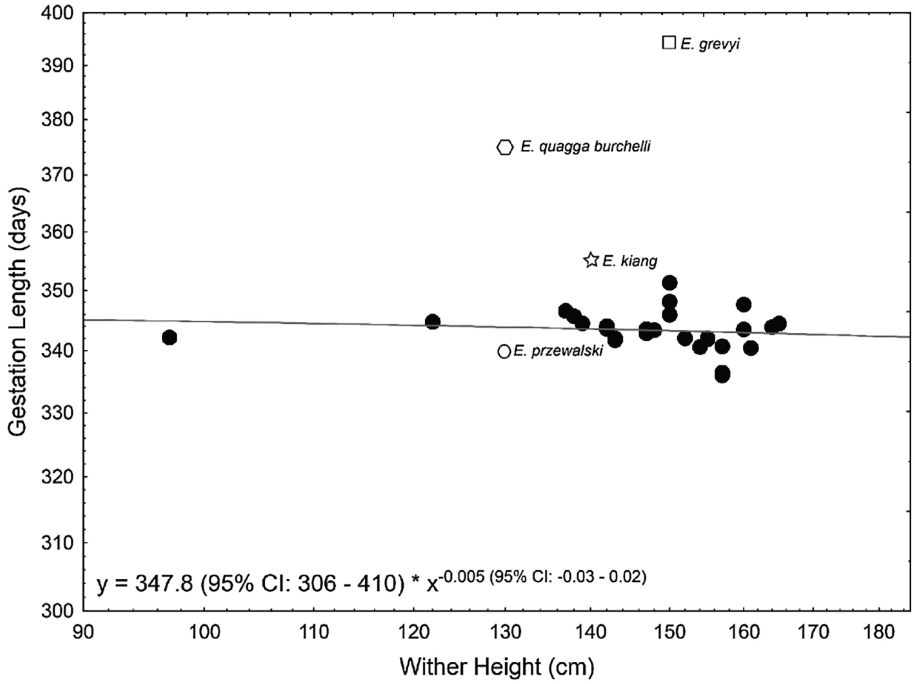

Fig. 2. Correlation of mean wither height and mean gestation length per breed (black circles) in a double log plot; additional data from the literature for wild equids (mean gestation length from different studies, see Table 1, white symbols); a regression line is displayed with the equation printed in the lower left corner.

jennies the difference between true gestation length and gestation length calculated from day of last mating can be around 10 days. In 99.8% of cases, mating occurs before ovulation (Davies Morel et al., 2002). Even after up to a week in the mare's genital tract, sperm is still be able to fertilize (Newcombe, 1994). To determine the day of ovulation, it is necessary to conduct daily examinations using ultrasonic scanning. The difference between the true gestation length and the perceived gestation length thus accounts for some variation among different studies and reviews. Given that we calculated gestation length for all our individuals equally, the variation of our dataset cannot be caused by methodological differences.

Variation in gestation periods could be linked to variation in body size even within breeds; no body size proxy for the individual records was available in our dataset. When comparing between breeds, the breed-specific standard withers height can be considered a valuable proxy that captures differences in body size between breeds reliably; differences between body types (e.g., typically heavily-muscled breeds vs. typically slim breeds) are, however, not represented by this proxy.

In our first hypothesis, we predicted significant differences among breeds, since previous studies on horse breeds showed variation in mean gestation lengths (Bos and Van der Mey, 1980; Langlois and Blouin, 2012; Valera et al., 2006). The results of the ANOVA support our hypothesis by showing significant differences among the 25 horse breeds with a variation of 11 days in mean gestation length. This result agrees with reviewed data from Satué et al. (2011), who listed differences of up to 13 days among mean gestation lengths of different breeds. The authors, however, argued that differences among various studies in their review might not be caused by breed but by differences in climate, geographical region, or photoperiod. Since we removed photoperiodic effects by calculating a linear model including month of insemination and collected our data in Central Europe, we can reject these variables as having affected our results. We further included foal sex in our linear model to adjust each gestation length accordingly, since previous studies reported foal sex to have a strong influence on gestation length with an increased gestation length in colt pregnancies (see Introduction). As we excluded two of the major influences by adjusting our data, the result of the ANOVA most likely present the variation related to breed affiliation.

Horses are seasonally polyestrus with breeding season starting in spring (McKinnon et al., 2011). Gestation length in seasonal breeders can be influenced by climate and photoperiod. To our knowledge no study exists which shows climate to have a significant influence on gestation length (Aoki et al., 2013; Bene et al., 2014; Bos and Van der Mey, 1980). Photoperiod, however, has been described as the main reason for seasonality (Caldas et al., 1994; Fitzgerald and McManus, 2000) as an increase in daylight hours triggers the transition from anestrus to cyclicity (Palmer and Guillaume, 1992). Various studies showed that artificial light treatment can advance the date of first ovulation after winter anestrus (Palmer and Driancourt, 1983; Scraba and Ginther, 1985). Furthermore, Cilek (2009) reported that gestation length decreases with increasing photoperiod. Hence differences in photoperiod or the application of artificial light at different stud farms might be a reason for variation in gestation length among breeds. All our data have been collected in areas with similar photoperiodic conditions. Thus, we disregard differences in photoperiod as cause for the variation in our dataset.

Including data collected from different stud farms, it is important to acknowledge that differences in management might also account for variation in gestation length. Langlois and Blouin (2012) found significant differences of ±1 day in gestation length among French horse breeders which they attribute to varying levels of breeding experience but declared negligible. On the contrary, differences depending on nutrition between war and post-war times of up to 4 days were presented by a different study (Uppenborn, 1933). The influence of nutrition was also confirmed for non-war times by Thorson et al. (2010), who reported differences in gestation length of 6.5 days between mares kept on pastures with or without supplemental grain feed. In our dataset, we cannot account for possible differences caused by management or nutrition, due to

the lack of data about the stud farms. Variations in management or nutrition could therefore have an influence on the different breeds and cause some of the variation found in our data.

Another major factor which is still in dispute is the influence of the mare. Some studies state that age of the mare has no significant effect on gestation length (Rezac et al., 2013; Winter et al., 2007), while others report a significant decrease of gestation length with increasing age of the mare. This decrease is supposed to be caused by a decrease in uterine and placental nutritional efficiency or hormonal differences at increasing age (Bos and Van der Mey, 1980; Demirci, 1988; Gluckman and Hanson, 2004; Meliani et al., 2011; Pashen and Allen, 1979). Parity as well as the reproductive states of the mare have been reported as an influence on gestation length by various studies (see Introduction). Furthermore, it has been reported that the mare can regulate the size of the foal (Allen et al., 2002) and suggested that gestation length can be prolonged by the mare by an embryonal diapause between day 20 and 40 (Lofstedt, 1993). The magnitude of the influence of other individual characteristics of mares on gestation length is yet to be determined. Studies on embryo transfer from one breed to another, for example, showed that foals of the larger breed have a lower birth weight than their siblings without embryo transfer if they were carried by the smaller breed and vice versa (Tischner, 1985; Wilsher and Allen, 2003). These differences in birth weight demonstrate that the mare has an influence on the foal. Due to the high sample sizes of our study, it appears reasonable to suspect that each breed is equally represented by mares of all ages, parities, and reproductive states in our study. Therefore, an influence of the mare as a cause for the variation among breeds in our study appears unlikely, but cannot be ruled out with certainty.

Our dataset included five breeds for which we also found values of mean gestation length in the literature (Table 1). In Arabs, our dataset resulted in a mean gestation length of 342 days and literature results range from 330.42 (Valente et al., 2006) to 340.3 (Valera et al., 2006) days. For Freiberger (341.9 days) only one comparing study could be found with an average gestation length of 336.5 days (Giger et al., 1996). The average gestation length of Friesians (340.5 days) ranges from 331.6 days (Sevinga et al., 2004) to 337.7 days (Bos and Van der Mey, 1980). In Haflinger (341.7 days) values from 337.8 days (Matassino, 1962) to 341.3 days (Bos and Van der Mey, 1980) could be found. Furthermore, the mean gestation length of Shetland ponies (342.1 days) ranges from 333.3 days (Walton and Hammond, 1938) to 337.2 days (Bos and Van der Mey, 1980). In all five cases, the mean gestation length of our study is slightly higher than the highest average stated by literature. This can be explained by the adjustment for sex and season which we included in our dataset.

Reported gestation lengths of wild equids range from around 330 days in Przewalski horses (Maltzan et al., 2007; Monfort et al., 1991) to 425 days in Grevy's zebra (Asa et al., 2001). Thus, reported gestation lengths on domesticated horses showed a larger variation ranging from 294 days (Rossdale, 1976) to 419 days (West, 1994). Although horses show this large variation, the majority of foalings occur between 325 and 368 days (Davies Morel et al., 2002). More data on wild equids are required to understand how domestication affected life history characteristics such as gestation periods in equids.

Our second aim for this study was to investigate the relationship between body size of a breed and its gestation length. Body size is known to account for most of the variability in metabolism (Brown et al., 2004; Speakman, 2005) and some variation in life history traits (Sibly et al., 2012) but does not correlate with gestation length among closely related species or even within species (Clauss et al., 2014). Thus, we hypothesized that gestation length does not correlate significantly with body size. The result of our allometric linear regression supports our hypothesis: it does not show a significant correlation between mean wither height and mean gestation length in horses. It is congruent with studies on other domesticated mammals such as dogs (Kirkwood, 1985), cattle (Andersen and Plum, 1965), and sheep (Bradford et al., 1972), where different sized breeds show similar gestation lengths. As Shetland Ponies (with a wither height of 97 cm) have a birthweight of 13.3% of their adult body weight and Shire Horses (with a wither height of 205 cm) have birth weight of 6.8% of their adult body weight, with both having a similar gestation length (Platt, 1984), it is evident that differences in absolute birth weight are caused by differences in intrauterine growth rate rather than the duration of growth.

Conclusion

Our study showed that the affiliation with breed is one of the factors leading to a large variation in gestation length in horses, whereas body size does not affect gestation length. Parturition in horses is difficult to foresee due to its high variability and unclear indicating signs (Meliani et al., 2011). This causes risks to mare and foal, leading to higher veterinary costs for breeders (Rezac et al., 2013). An increased understanding of the influences on gestation length could help stud farms to decrease the risks associated with foaling and to increase breeding productivity; it could also help to understand the selection pressures that led to potential modifications of gestation length during the process of domestication and breed formation.

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Chapter II

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Short communication

Do domesticated mammals selected for intensive production have less variable gestation periods?

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ABSTRACT

The variability of life history traits is affected by domestication. As gestation length is an important life history trait for production management, its variability is hypothesized to be lower in highly controlled production animals. Furthermore, some authors claim that horses have a particularly variable gestation length compared to other domesticated mammalian species. To test this, we compared 192 gestation lengths from the literature for eight different mammalian species. In this sample, gestation length does not contain a phylogenetic signal. Instead, production animals display lower variation than non-production animals. Horses fall well within the range of variation of gestation length in other domesticated companion animals.

The process of domestication can alter life history traits (Geiger et al., 2016; Herre and Röhrs, 1990; Kihlström, 1972) and affects the variability of these traits. This can be particularly evident in cases where there are clear selection goals as in cases of intense and directed domestication. If a trait is of little importance, then its variability is probably high, whereas if the trait is important, then variability should be low (Darwin, 1859).

One of the life history traits affected by domestication is gestation length (Heck et al., 2017). As reproduction is important in production animals such as cattle, sheep, and pigs (where one could easily hypothesize that economic pressures favor the selection of short gestation periods, i.e. fast reproduction), one could expect production animals to have less variability in this life history trait than animals bred for non-production purposes, such as most horse breeds or dogs. The gestation length in horses allegedly shows a larger variation than in other domesticated mammal species (Aoki et al., 2013; Bos and Van der Mey, 1980). Bos and Van der Mey (1980) concluded this after finding that the coefficient of variance (CV) for the gestation lengths in horses [2.8 to 3.7] was higher than that established for cattle [1.5] (Banerjee-Schotsman, 1964), sheep [1.6] (Prud'hon et al., 1970), and pigs [1.4] (Cox, 1964).

We aimed to investigate the differences of variation in gestation length among different domesticated mammal species, as suggested by some studies on cattle or goats (Asdell, 1929; Brakel et al., 1952). We tested the hypothesis that more strictly controlled production animals have less variation in their gestation length than non-production animals. As in any comparison across taxa, in this case encompassing a large portion of the placental mammal tree of life, the effect of phylogeny should be considered. Changes in anatomical and life history traits associated with domestication are known to be affected by the degree of relatedness among the species considered (Francis, 2015; Sánchez-Villagra et al., 2016). Furthermore, the variation in anatomical or life history traits can be phylogeny-dependent (Asher et al., 2011). Previous research has shown that gestation lengths scale differently with size depending on the taxonomic level (Clauss et al., 2014).

We gathered literature data on 192 mean gestation lengths and their standard deviations (SD) from domesticated forms of eight species (Table 1, Supplement Table 1). The average sample size was 24 recorded mean gestation lengths; species with three or more records of mean gestation lengths were included in the analysis. In studies where more than one mean was presented per breed, we used the data with the largest sample size. Furthermore, we included data from veterinary studies where parturition was, for example, induced by different hormonal treatments or embryo transfers were conducted. From these veterinary studies, we only used the datasets marked as control. In some research articles, the mean was presented with the standard error (SE). In these cases we used the sample size (n) and the SE to calculate the SD [$\sqrt{n}$*SE]. To compare the different species, we calculated the coef-

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Table 1
Details of literature sources for specimens in Supplementary Table 1.

Animal	Literature Source
Cat	Platz et al. (1978), Pope et al. (2009), Root et al. (1995), Sparkes et al. (2006)
Cattle	Akkayan and Ada (1975), Brakel et al. (1952), Burris and Blunn (1952), DeFries et al. (1959), Dessouky and Rakha (1961), Fitch et al. (1924), Knott (1932), Piedrafita et al. (2000), Rendel (1959), Sabatini (1908), Stallcup et al. (1956), Tessier (1817), Ward and Castle (1947)
Dog	Chakraborty (1987), Concannon et al. (1983), Eilts et al. (2005), Krzyzanowski et al. (1975), Kutzler et al. (2003), Okkens et al. (1993), Shimatsu et al. (2007), Tsutsui et al. (2006)
Goat	Amoah et al. (1996), Asdell (1929), Greyling (2000), Mellado et al. (2000), Talukder et al. (2016)
Horse	Ali et al. (2014), Aoki et al. (2013), Bene et al. (2014), Cilek (2009), Davies Morel et al. (2002), Dicken et al. (2012), El-Wishy et al. (1990), First and Alm (1977), Heck et al. (2017), Heidler et al. (2004), Hrasnica (1944), Hura et al. (1997), Matassino (1962), Mauch (1937), Meliani et al. (2011), Pérez et al. (2003), Pozo-Lora (1954), Rezac et al. (2013), Rosales et al. (2017), Sabatini (1908), Salerno and Montemurro (1966), Talluri et al. (2016), Taveira and da Mota (2007), Tessier (1817), Valera et al. (2006), Villani and Romano (2008), Winter et al. (2007)
Pig	Baxter et al. (2008), Chidgey et al. (2016), Cox (1964), Diehl et al. (1974), Farkas et al. (2007), Hanenberg et al. (2001), Kennedy and Moxley (1978), Omtvedt et al. (1965), Rydhmer et al. (2008), Sabatini (1908), Sasaki and Koketsu (2007), Van Dijk et al. (2005)
Rabbit	Ewuola et al. (2016), Lukefahr and Hamilton (1997), Rosahn et al. (1935)
Sheep	Alexander (1956), Bradford et al. (1972), Cleal et al. (2007), Fogarty et al. (2005), Forbes (1967), Ford et al. (2007), Osinowo et al. (1993), Öztürk et al. (2016), Roda and Otto (1989), Sabatini (1908), Smith et al. (2008), Tessier (1817), Thompson et al. (1995), West (1996), Zarkawi (1997)

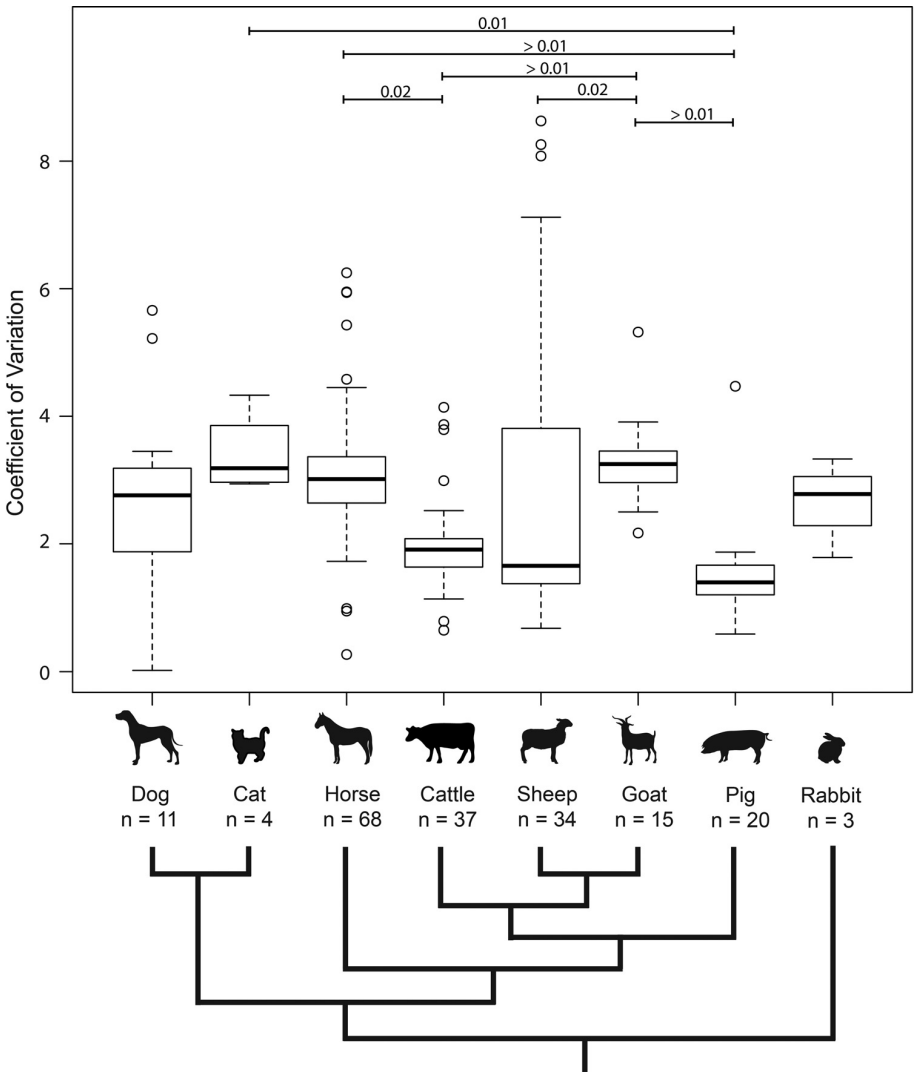

Fig. 1. Comparison of gestation length variation among domesticated forms of eight mammal species; CV = coefficient of variance; n = sample size; the bold black line shows the median and the bars denote the non-outlier range; the tree is based on the tree from Sánchez-Villagra et al. (2016), O'leary et al. (2013), and Bibi (2013) with the length of the branches not being time calibrated; the black lines above show the significant comparisons of the post-hoc Tukey test.

ficient of variance [(SD*100)/Mean], which is an informative value independent of the differences in average gestation length. We evaluated differences in gestation length among species using a non-parametric Kruskal-Wallis test followed by a Tukey post-hoc test. Although we consider that variation in life history traits can be phylogeny-dependent, the limited data on gestation length of

domesticated mammals do not allow for phylogenetic analyses, so phylogenetic considerations had to be applied in a qualitative manner. All statistics were performed using the functions Kruskal.test and posthoc.Kruskal.nemenyi.test (Rpackage PMCMR, version 4.1, Pohlert, 2014) in RStudio (Version 1.0.136, RStudio Inc. Team R, 2017). The significance for all tests was set at alpha = 0.05.

There were significant differences in the CV among the eight species of domesticated mammals (Kruskal-Wallis test: chi-squared = 45.527, df = 7, p-value ≤ 0.001, Fig. 1). For the pairwise comparisons 6 out of 28 were significant (post-hoc Tukey test, Fig. 1). Cats, horses and goats have a significantly higher CV than pigs (cats*pigs $p = 0.015$; horses*pigs $p \leq 0.001$; goats*pigs $p \leq 0.001$). Horses and goats show a significantly higher CV than cattle (horses*cattle $p = 0.023$; goats*cattle $p = 0.0027$). Goats also show a significantly higher CV than sheep ($p = 0.0191$). Within the closer related species of the artiodactyls, we found three of the six significant pairwise comparisons, which suggest that phylogeny has little influence on the variation of gestation length in our study.

Gestation length has been hypothesized to be "relative immune to changes within species" (Promislow and Harvey, 1990). Domestication using artificial selection as an evolutionary mechanism, however, can alter life history traits, including gestation length in various ways. In strong artificial selection, the selection for a certain favorable trait, a shorter gestation length in production animals in this case, can be modified pre- and postzygotically (Driscoll et al., 2009). In cattle, for example, the use of genomic selection to aid breeding strategies has been part of reproduction management since the 1970s (Chebel and Ribeiro, 2016). Christie et al. (2016) showed that "a single generation of domestication can translate into heritable differences in expression at hundreds of genes". The underlying genetic bases of gestation length might be more variable in non-production animals than in production animals due to selective breeding.

Some breeders influence the reproduction of race horse breeds by using hormones and artificial light use (Bristol, 2000; Hodge et al., 1982; Nolan et al., 2017). Artificial light is used to induce ovulation by advancing the breeding season to mid-February. This is beneficial in breeding branches due to the artificial age determination in horses by which all foals are born on the 1st of January. This procedure, however, does not alter the gestation length itself. A shortening of the gestation length can be achieved by hormonal treatments, which are too expensive and labor intensive for regular usage (Bristol, 2000; Leadon et al., 1982).

We reject the hypothesis (Aoki et al., 2013; Bos and Van der Mey, 1980) that horses have a larger variation in their gestation length than other domesticated mammals. The intraspecific CV of the domesticated mammal species in our data set (dog = 2.76, cat = 3.18, horse = 2.92, cattle = 1.91, sheep = 1.66, goat = 3.25, pig = 1.4, rabbit = 2.78) fall within the range that has been reported for other mammalian species, which range from 0.4 in *Lama pacos* up to 8.7 in *Elephas maximus* (Kiltie, 1982).

Data on gestation length in various domesticated breeds are scanty and not evenly distributed among species. Our dataset is based on literature data with different sample sizes and different methods to calculate the used parameters, which might account for some of the variation found. The calculated CVs, however, are well within the ranges of other studies and we therefore are confident that the error introduced by the data collection in the different studies does not affect the conclusions we reach here.

With cattle, sheep, and pigs showing the lowest variation in gestation length, the hypothesis that stricter controlled production animals have lower variation in their gestation length than non-production animals is supported. Goats, also counting as production animals, are an exception. Asdell (1929) also reported less variation of gestation length in sheep than in goats and argued that goats have probably been less strictly selected than sheep. Although counting as production animals, goats are commonly kept in a more feral lifestyle with less production pressure than sheep (Herre and Röhrs, 1990; Mason, 1984). Thus, our study provides evidence that a long history of selective breeding for production efficiency may lead to reduced variation in gestation periods.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mambio.2017.09.004.

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Chapter III

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Shape variation and modularity of skull and teeth in domesticated horses and wild equids

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Abstract

Background: In horses, the morphological changes induced by the process of domestication are reportedly less pronounced than in other species, such as dogs or pigs – although the horses' disparity has rarely been empirically tested. We investigated shape differences and modularity of domesticated horses, Przewalski's horses, donkeys and zebras. Mandibular and tooth shape have been shown to be valuable features for differentiating wild and domesticated forms in some mammals.

Results: Both mandible and teeth, show a pattern of shape space occupation analogous to that of the cranium, with domesticated horses occupying a similar extension in shape space to that of wild equids. Only cranial shape data exhibit a tendency to separate domesticated horses and Przewalski's horses from donkeys and zebras. Maximum likelihood model-based tests confirm the horse cranium is composed of six developmental modules, as reported for placental mammals in general. The magnitude of integration in domesticated horse skull was lower than in wild equids across all six cranial modules, and lower values of integration were associated with higher disparity values across all modules.

Conclusion: This is the first study that combines different skeletal features for the description and comparison of shape changes in all living equid groups using geometric morphometrics. We support Darwin's hypothesis that the shape variation in the skull of domesticated horses is similar to the shape variation of all wild equid species existing today. Lower magnitudes of module integration are recovered in domesticated horses compared to their wild relatives.

Keywords: Domestication, Disparity, Modularity, Geometric morphometrics, Cranium

Background

After being on the verge of extinction, domestication made horses one of today's most common large animal species [1]. All living species of equids belong to the genus *Equus,* which is divided into the caballine taxa, including domesticated horses (H) and Przewalski's horses (P), and non-caballine taxa, comprising the different donkey (D) and zebra species (Z). Within the caballine taxa, the Przewalski's horses likely represent the sister-taxon to the extinct wild ancestor of domesticated horses [2, 3]. Since the early domestication of horses, reproductive isolation promoted divergence by genetic drift and natural selection. Later on, extensive selective breeding to meet human needs for certain behavioural or physiological traits resulted in a wide range of morphological variation [4–6]. Horses, like other domesticated species, have been shaped into diverse morphological types through artificial selection to fit specific functions, such as agricultural work, racing, or leisure. Four traditional body types are recognized: draft horses, medium horses, light horses, and ponies [5, 6]. Horse disparity was already acknowledged by Charles Darwin, who noted that its intraspecific disparity is larger than the interspecific disparity of equids in general [7]. Darwin proposed that great differences among horse breeds can be found in the skull. Based on its complexity in form and origin, as well as its relation to important vital functions, the skull is the most commonly used marker of morphological variation [8]. The increase in skull shape variation following domestication has been

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found in different domesticated species such as dogs [9], cattle [10], and pigeons [11], where it has been measured using geometric morphometric methods, and quantified through comparisons of variance in shape space. In addition, potential shape changes in teeth are commonly used in zooarchaeological studies to determine the time and location of domestication [12–16]. Previous studies on skull and tooth morphology and morphometrics show the existence of intraspecific as well as interspecific shape variation in subsets of the equid clade (Table 1). However, the patterning and magnitude of variation in skull shape or tooth shape across all extant equids has so far not been examined. In order to quantify the shape variation in extant equids and to investigate the impact of domestication, we first compare domesticated horses represented by 38 different breeds and encompassing the whole size range of the species, to the extant zebra and donkey species, as well as to the Przewalski's horse (Table 2) using two- and three-dimensional geometric morphometrics to explore cranial and mandible (3D), and teeth (2D) morphometrical variation.

As a second part of this study, we examine modularity of the equid skull. The concept of modularity [17, 18] has attracted much attention in recent years (e.g. [19–26]), having emerged as a quantitative framework for exploring questions relating to facilitation and constraint in morphological evolution, with the goal of understanding how (and by how much) the direction in which variation is generated is biased [27–29]. Many studies have quantified patterns of modularity in the cranium using inter-trait correlations extracted from geometric morphometric data (see [30] and references therein) and, taken together, their results have supported a

Table 1 Overview of previous literature on skull and/or tooth morphology and morphometrics in extant equids

Author	Species and/or breeds	Body part	Method	Summary
Bennett (1980) [38]	*Equus andium, E. asinus, E. burchelli, E. caballus* (including *E. caballus alaskae,* originally named *E. niobrarensis alaskae* by Hay, 1915), *E. calobatus, E. conversidens, E. francisi, E. grevyi, E. hatcheri, E. hemionus, E. kiang, E. onager, E. occidentalis, E. quagga, E. scotti, E. zebra,* and *Dinohippus*	Skull & teeth	Descriptive morphology	Living species of *Equus* can be differentiated by a number of morphological characters
Seetah et al. (2016) [14]	Icelandic, Thoroughbred, Przewalski's horses, and potentially *E. ferus*	Teeth	2D geometric morphometrics	Tooth shape of horses largely resembles those of Pleistocene and recent wild horses until the onset of modern breeds
Seetah et al. (2014) [48]	Icelandic and Thoroughbred horses	Teeth	2D geometric morphometrics	Significant differences between the two horse breeds in tooth shape
Evans & McGreevy (2006) [63]	Thoroughbreds, Standardbreds, Ponies, Arabs, Anglo-Arabs, Quarter horse, Warmblood, and Appaloosa	Skull	Classic morphometrics	No overall shape differences exist but modular differences (nasal vs. cranial)
Zhu et al. (2014) [64]	*E. asinus* compared to ponies from Jie (1995) and Evans & McGreevy (2006)	Skull	Classic morphometrics	Supports the two modules from Evans & McGreevy (2006) and shows that donkeys have a longer nasal part
Hanot et al. (2017) [39]	Domestic horses (*E. caballus*) of various breeds (i.e. racehorses, draft horses, Shetland ponies, Icelandic ponies, Camargue horse, Pottok, Konik), Przewalski's horses (*E. przewalskii*), domestic donkeys (*E. asinus asinus*) and wild asses (*E. a. africanus*), mules (*E.asinus x E. caballus*) and hinnies (*E. caballus x E. asinus*)	Skull & skeleton	3D geometric morphometrics	Occipital part of the skull is especially discriminant among species and it is possible to identify domesticated equids from archaeological sites
Cucchi et al. (2017) [16]	*E. ferus caballus, E. f. przewalskii, E. africanus somaliensis, E. a. asinus, E. kiang, E. hemionus hemionus, E. h. khur, E. h. kulan, E. grevyi, E. zebra hartmannae, E. quagga quagga, E. q. burchelli,* and hybrids (donkey*horse)	Teeth	2D geometric morphometrics	Enamel folding is a good phylogenetic marker; strong taxonomic pattern is visible in enamel folding
Eisenmann & Baylac (2000) [65]	*E. grevyi, E. burchelli boehmi, E. zebra zebra, E. asinus, E. h. kulan, E. przewalskii,* and *E. caballus*	Skull	Classic morphometrics	Domestic horses and Przewalski's horses can be differentiated

Table 2 Number of individuals in each group (domesticated horses (H), Przewalski's horses (P), donkeys (D), and zebras (Z)) on each cranium, mandible, and tooth; sample of domesticated horses is present by breed

Group	Breed	Cranium	Mandible	U3M	U2P	L3M	L2P
Domesticated horses (H)	Ancient breed	2	1	4	3	1	1
	Anglo-Norman	2	2	1	2	2	2
	Arab	7	8	6	7	4	2
	Birkenfelder	1	1	1	0	0	0
	Belgian Draft	11	10	9	8	6	9
	Bosnian Pony	1	0	0	0	0	0
	Clydesdale	3	3	3	3	3	2
	Exmoor Pony	1	1	1	1	1	1
	Falabella	1	0	0	0	0	0
	Galician Farm Horse	3	3	2	3	2	2
	Grisons (Graubünden)	3	3	3	3	3	3
	German Riding Pony	2	2	1	2	0	2
	Hannoverian	2	3	2	3	1	1
	Hackney	2	2	2	2	1	1
	Holstein	1	1	1	1	1	0
	Hungarian	3	3	3	3	1	3
	Huzule	3	2	2	2	2	1
	Icelandic Horse	16	18	17	18	12	16
	Indian Pony	2	1	2	2	1	1
	Kladrubian	10	10	10	10	9	6
	Konik	1	1	0	1	0	0
	Kosarian	1	1	1	1	0	1
	Lipizzan	2	2	0	2	0	2
	Mongolian	3	3	3	1	2	1
	Norik	2	2	2	1	1	0
	Oldenburgian	1	1	1	1	1	1
	Pinzgau	18	17	16	17	15	9
	Polish Farm Horse	1	1	1	1	1	1
	Scottish Pony	2	1	1	1	0	0
	Seneca Sarajevo	1	0	1	1	0	0
	Shetland Pony	6	6	5	5	4	5
	Shire	1	1	1	1	1	0
	Styrian	1	1	1	1	1	0
	Suffolk	2	2	1	2	1	1
	English Thoroughbred	6	7	6	7	6	5
	Togo Pony	0	5	4	4	2	3
	Trakehner	3	4	4	4	3	4
	Welsh	6	12	4	6	4	7
Subtotal		133	141	122	130	92	93
Donkeys (D)		31	33	25	24	20	18
Zebras (Z)		47	48	42	41	29	28
Przewalski's Horses (P)		5	2	3	3	3	2
	Total	216	224	192	198	144	141

common pattern in therian mammals, with some variability in the magnitude of integration among species (e.g. [20, 31]). In contrast, comparatively little is known about the lability of modular patterning and integration magnitudes on relatively short time scales and under selective breeding regimes, although changes in magnitude, rather than patterning, have been implicated as the target for selection [32]. Providing examples of selective breeding for features to suit human activities, the study of domestication events offers an opportunity to empirically examine the role of modularity and integration in the generation of cranial disparity over short evolutionary timescales. A modular structure of the skull is expected to be uncovered for horses, as has been found across a wide spectrum of mammals (e.g. [31]), and we assess the fit of our shape data to functional and developmental hypotheses for modular patterning [33] that have been previously tested in the mammalian cranium. According to Darwin's hypothesis, domesticated horses should show more variation in shape than the wild equid species. If this hypothesis is supported, then we should find differences in integration and disparity measures for cranial modules between wild equids and domesticated horses. To do so, we assess whether cranial modules display a) higher or lower magnitudes of integration and b) high or low disparity for domesticated horses and wild equids, and c) we investigate whether there is a relationship between module integration/disparity and regions of the cranium showing most variability in shape among domesticated horses.

Our aim is to characterize and quantify the patterning and magnitude of shape variation in the skulls and teeth of domesticated horses compared to the wild species of *Equus*. We use geometric morphometric methods to: a) test Darwin's hypothesis that the magnitude of intraspecific disparity in horses is larger than the interspecific disparity in equids, b) examine the extent to which domestication influenced tooth shape in equids, c) investigate whether the patterning of shape variation in horse skulls reflects a modular structure, specifically identifying the model best supported for the landmark data by evaluating four modular hypotheses that reflect developmental and functional trait interactions in the cranium, and d) quantify differences in the magnitude of modularity and integration between domesticated horses and wild equids.

Given the well-documented palaeontological record of horses [34], these animals offer the possibility to compare diversification in macroevolutionary and microevolutionary scale like few others. In fact, previous classic studies by Radinsky [35, 36] investigated some of the cranial transformations with morphometric approaches typical of that time. Our study expands the studies of the extant species using newer morphometric approaches and provides the basis for future works comparing also the fossil record of the clade.

Results

Shape variation

In the cranial symmetrised shape data, the first three principal components (PCs) account for 43.1% of the total shape variation in the cranium (Fig. 1a). PC 1 (17.6%) tends to separate the caballine equids (H, P) from the non-caballine equids (D, Z). The shape change along PC 1 from negative to positive is dominated by a narrowing and straightening of the skull in combination with an elongation of the occipital-parietal region, represented by the cranial vault (Fig. 1b). PC 2 accounts for 15.2% of the overall observed variation, and is characterized by a general broadening of the skull in combination with an elongation of the occipital-parietal region (cranial vault module) and a shortening of the nasal region (anterior oral-nasal module). Because of the large number of landmarks compare to the relatively small number of specimens we applied a dimensionality reduction of the datasets by selecting the first PCs for all further analyses following Evin et al. [13] (mevolCVP function) that also takes into account unbalanced sample size between groups. The results of the mevolCVP function suggested the reduction of the dataset to the first three PCs in all further analyses for the cranial data. Significant differences among the four groups (Procrustes ANOVA $p < 0.001$, F = 35.578, based on the 6 first PCs) allowed us to perform a canonical variance analysis (CVA) with a-priori defined groups (H, D, P, Z) resulting in an overall classification accuracy of 78.4% (Confidence interval CI: 60%–95%) when the four groups are analysed, and 98.2% (CI: 96.8%–100%, based on the 17 first PCs, Procrustes ANOVA $p < 0.001$, F = 32.723) when the Przewalski horse specimens are excluded. In this later analysis, both domestic horses and donkeys could be correctly assigned to their respective groups in 100% of the cases. Zebras were assigned correctly for 95.7% of cases and the remaining 4.3% were grouped within the donkeys. Predictive discriminant analyses detect cranial shape proximities of the five Przewalski's horses with the domestic horses (100% probabilities of identification). Domesticated and Przewalski's horses are most distinct from donkeys and zebras in Procrustes shape space, as measured by Mahalanobis distance (Table 3). Horses occupy a larger Procrustes shape space (53.12%) as determined by Foote's partial disparity, than zebras (26.22%) and donkeys (18.34%). The Przewalski's horses occupy only 2.26% of the overall shape space. The overall Procrustes variance for the cranium is 0.0023.

The first four PCs of the mandible shape data account for 64.1% of the total shape variation in the mandible. In contrast to the cranium, none of the PCs shows separation between any of the four groups. Specimens of all groups largely overlap in PC shape space (Fig. 1c and d), therefore we do not discuss this further. Due to

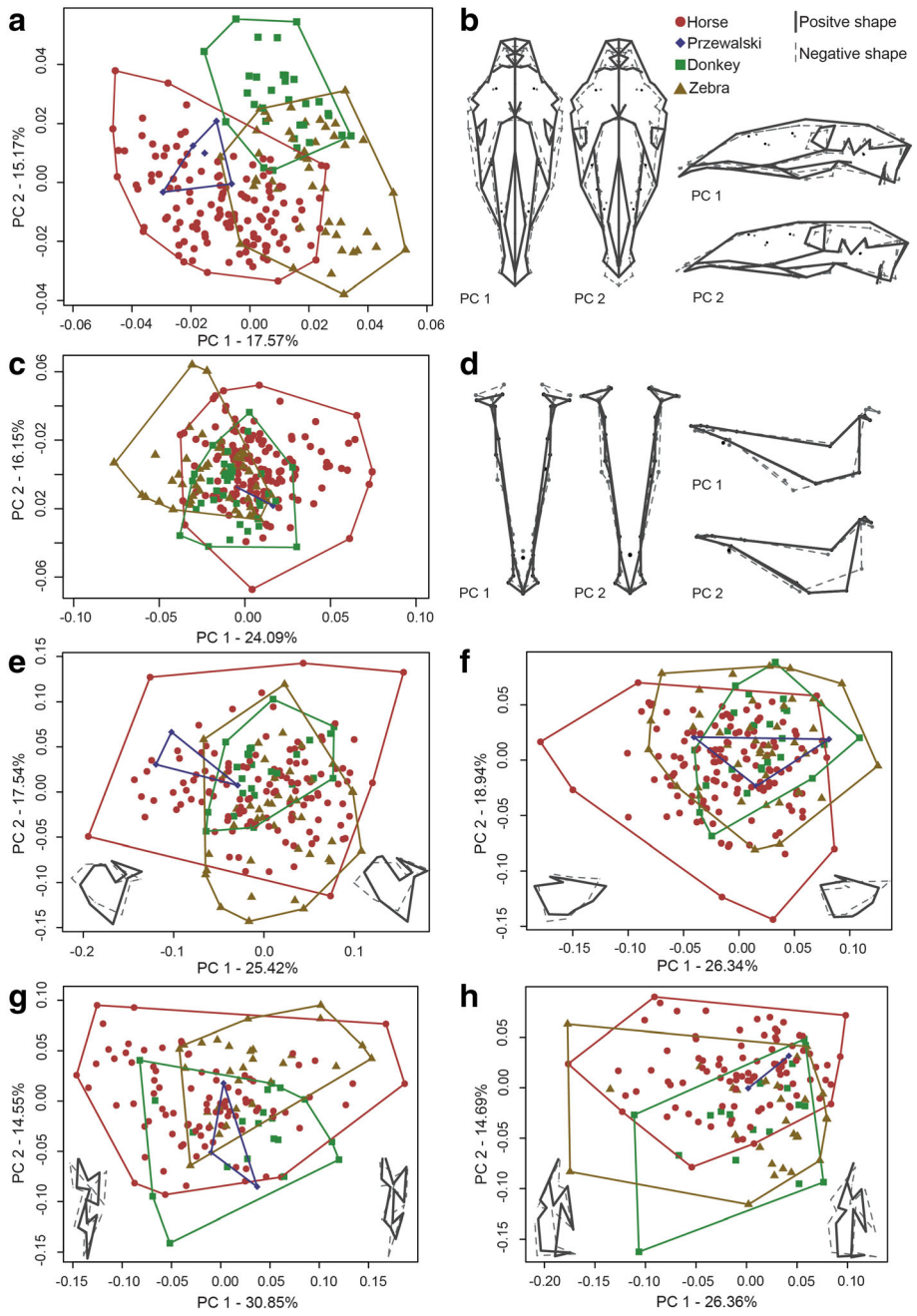

Fig. 1 Principal component analysis of **a** the cranial landmark data of 216 adult equid specimens: zebras ($n = 47$), donkeys ($n = 31$), Przewalski's horses ($n = 5$), and domesticated horses ($n = 133$), **b** Shape changes in dorsal and lateral view for PC 1 and PC 2 with black lines presenting positive shape and dotted, grey lines presenting negative shape, **c** the mandible landmark data of 224 adult equid specimens: zebras ($n = 48$), donkeys ($n = 33$), Przewalski's horses ($n = 2$), and domesticated horses ($n = 141$), **d** Shape changes in dorsal and lateral view for PC 1 and PC 2 with black lines presenting positive shape and dotted, grey lines presenting negative shape, **e** of the upper 3rd molar landmark data of 225 adult equid specimens: zebras ($n = 42$), donkeys ($n = 25$), Przewalski's horses ($n = 3$), and domesticated horses ($n = 122$), **f** the upper 2rd premolar landmark data of 225 adult equid specimens: zebras ($n = 42$), donkeys ($n = 25$), Przewalski's horses ($n = 3$), and domesticated horses ($n = 122$), **g** of the lower 3rd molar landmark data of 225 adult equid specimens: zebras ($n = 29$), donkeys ($n = 20$), Przewalski's horses ($n = 3$), and domesticated horses ($n = 92$), **h** of the lower 2rd premolar landmark data of 225 adult equid specimens: zebras ($n = 29$), donkeys ($n = 20$), Przewalski's horses ($n = 3$), and domesticated horses ($n = 92$); Symbols are circles: domesticated horses, diamonds: Przewalski's, triangles: zebras, and squares: donkeys

Table 3 MAHALANOBIS DISTANCE

	Cranium			Mandible		
	D	H	P	D	H	P
H	5.67			10.08		
P	5.27	1.04		6.81	2.66	
Z	2.32	6.37	6.31	5.6	9.55	7.81
	Cranium 3M (U3M)			Cranium 2P (U2P)		
	D	H	P	D	H	P
H	1.77			2.56		
P	2.61	1.95		2.82	1.61	
Z	1.83	1.58	2.79	1.85	2.52	3.13
	Mandible 3M (L3M)			Mandible 2P (L2P)		
	D	H	P	D	H	P
H	2.23			2.16		
P	2.33	1.84		2.42	0.96	
Z	2.79	2.02	3.37	1.27	2.32	2.51

significant results of the Procrustes ANOVA ($p < 0.001$, F = 14.4, 3 first PCs), we computed a CVA with the same a-priori groups used in the cranium. The overall classification accuracy was low when the four groups were analysed (H, P, D, Z) 38.4% (CI: 12.5%–87.5%), while a classification accuracy of 87% (CI: 82.4%–91.2%, 17 first PCs, F = 15.681, $p < 0.001$) was reached when the two Prezwalski horses were excluded. In this later analysis, 88.2% of the donkeys, 87.9% of the horses and 89.6% of the zebras were correctly classified. The two Przewalski's specimens were both classified as horses with probabilities of 100% and 72%. Donkeys, zebras, and Przewalski's horses are similarly spaced from each other (Mahalanobis distance, Table 3). Mandible Procrustes shape space occupation is very similar to the cranial shape space with horses dominating the shape space (Foote's partial disparity 58.98%). Zebras occupy the second largest shape space with 23.69%, followed by donkeys (16.59%) and Przewalski's horses (0.71%). The Procrustes variance of the mandible (0.0024) is slightly higher than in the cranium.

In the teeth of the upper tooth row, the first four PCs of the third molar (U3M) account for 66.6%, and of the second premolar (U2P) for 70.1% of the shape variation. In the lower tooth row, only the first three PCs are each above 10% and account for 55.8% in the third molar (L3M) and 51.5% in the second premolar (L2P). None of the PCs show separation of the four groups from each other for any of the teeth, with specimens of all groups largely overlapping in PC shape space (Fig. 1e-h). The four groups differ in the shape of their four teeth (U3M: $p < 0.001$, F = 2.8609, 2 first PCs; U2P: $p < 0.001$, F = 9.0841, 4 first PCs; L3M: $p < 0.001$, F = 6.1919, 4 first PCs; L2P: $p < 0.001$, F = 4.4724, 7 first PCs). We computed CVAs with the same a-priori groups (H, P, D, Z) before removing the smallest group of Przewalski horse, like for the cranium and mandible analyses. The overall classification accuracy was similar among all teeth with 41.3% (CI: 24.6%–66.7%, 6 PCs) for U3M, 39.4% (CI: 16.7%–66.7%, 2 PCs) for U2P, 35% (CI: 8.3%–58.3%, 4 PCs) for L3M, and 34.5% (CI: 11.9%–62.5%, 7 PCs) for L2P. For the later comparison excluding the Przewalski's specimens, overall classification accuracy was similar among all teeth: 63.9% (CI: 56%–70.7%, 11 PCs) for U3M, 76.4% (CI: 69.4%–83.3%, 12 PCs) for U2P, 75% (CI: 66.6%–81.7%, 8 PCs) for L3M and 70.9% (CI: 63%–79.7%, 9 PCs) for L2P. The Przewalski's specimens show close shape proximities with horses for L2P (two specimens identified to horses with probabilities of 100% and 96.7%) and U2P (three specimens with probabilities between 91.7% and 95.8%), while for L3M two of the three specimens were closer to donkeys (65.8% and 100%) and the latest to horse (100%), and for U3M for which two specimens were identified as close to horses (100% and 52.9%) and one to donkey (100%).

All analysed teeth (L2P, U2P, L3M, and U3M) have a similar partial disparity as all other analysed features: horses showing the highest partial disparity followed by zebras, donkeys, and Przewalski's horses. However, the overall disparity (Procrustes variance) differs between the teeth, with cranial P2 showing the smallest variance (0.008) while all other teeth exhibit a total variance around 0.013. The Mahalanobis distances among the groups calculated for each tooth separately are similar for cranial and mandibular P2, and cranial and mandibular M2. Przewalski's horses and zebras are in all instances most disparate. The P2 is most similar between horses and Przewalski's horses, the cranial M3 is most similar between zebras and horses and the mandibular M3 is most similar between Przewalski's horses and horses followed by the zebra (Table 3).

Modularity

Results from EMMLi indicated that, of the models tested here, the best supported model for modularity was Goswami's mammalian module hypothesis [20] with separate within-module integration and separate between-module integration (model 2d, Additional file 1: Table S1). This model had the lowest Akaike Information Criterion (AICc) value of − 969.34 and a maximum likelihood of 507.97 (Additional file 1: Table S1). Goswami's mammalian module hypothesis contains six modules, these are anterior oral-nasal (AON), cranial base (CB), cranial vault (CV), orbit (ORB), molar (MR), and zygomatic-pterygoid (ZP) (Fig. 2, [20]). Disparity and integration values were calculated for these six modules separately for domesticated horses (H) and wild equids (P/D/Z).

Eigenvalue dispersion values indicated that each of the six modules showed lower magnitudes of integration in domesticated horses (average = 0.68, median = 0.72) compared to wild equids (average = 0.73, median = 0.79). For domesticated horses, eigenvalue dispersion values were lowest for AON (0.53) and highest for MR (0.81) (range = 0.28). For wild equids, integration values were lowest for ZP (0.58) and highest for MR (0.84) (range = 0.26) (Additional file 2: Table S2). The ZP module was most similar in terms of magnitude of integration between domesticated horses and wild equids (difference of 0.03, 4.7%), whereas the CB module had the highest integration in wild equids compared to domesticated horses (difference of 0.07, 9.5%, Additional file 2: Table S2). Module disparity values were higher in domesticated horses compared to wild equids for four out of six modules; these were AON, CV, MR, and ZP (Additional file 2: Table S2). Average disparity across all six modules was the same for both domesticated horses and wild equids (0.034), with disparity ranging from 0.026 (MR) to 0.046 (ZP) for domesticated horses (median = 0.031) and from 0.021 (MR) to 0.041 (ORB) for wild equids (median = 0.035). The ORB module showed the largest difference in disparity between the two groups. Disparity values for the AON module were most similar for domesticated and wild forms (Additional file 2: Table S2). In both wild equids and domesticated horses there is a general trend of increasing disparity with decreasing magnitude of integration. Further, the AON and ZP modules stand out from the other cranial modules as they both show higher disparity coupled with lower integration values (Fig. 2).

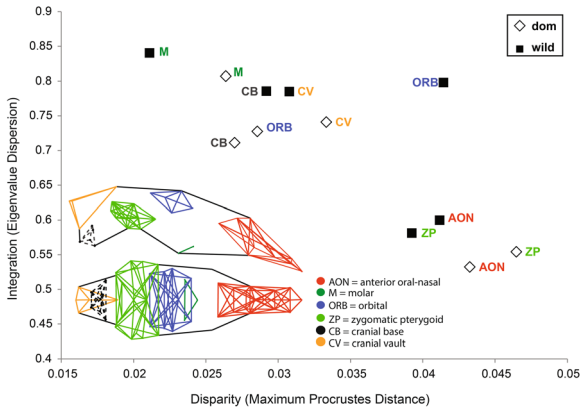

Fig. 2 a Scatterplot of calculated values for disparity (x-axis) and integration (y-axis) for all modules and wild horses and domesticated equids separately; **b** Six cranial modules in a schematic horse skull (black outline) after Goswami [20] shown in lateral and dorsal perspective; Modules are: anterior oral-nasal (AON, red), molar (M, dark green), orbital (ORB, dark blue), zygomatic pterygoid (ZP, light green), cranial base (CB, orange), and cranial vault (CV, black dashed)

Discussion

The results of our study on skull shape variation support Darwin's hypothesis that the intraspecific disparity in horses is larger than the interspecific disparity in equids. The shape variation of domesticated horses is not only larger than that of the closest relative (Przewalski's horses) but similar to the shape variation of all the wild equid species existing today. Horses do not only dominate the Procrustes shape space when comparing crania, but also comparing mandibles or teeth - showing higher shape variation in all tested elements.

The overall classification among domesticated horses (H), Przewalski's horses (P), donkeys (D), and zebras (Z) had an average accuracy of 44.5% (range: 34.5% - 78.4%), which lies significantly above the random chance accuracy of 25%. When we excluded the Przewalski's specimens due to their small sample size the average accuracy increased to 89.2% (range: 63.9% - 98.2%). The separation of caballine from non-caballine taxa and the clustering within these sister clades of H & P, and D & Z by the Mahalanobis distances, is in accordance with the phylogenetic relationship of equids. All four groups descend from a common ancestor around 4–4.5 myr BP, with zebras and donkeys splitting around 2.8 myr BP and Przewalski's horses and the wild ancestor of today's domesticated horses splitting 38–72 kyr BP [2, 3, 37]. The first PC of the cranial shape data tended to separate caballine from non-caballine taxa. The accompanying shape differences are dominated by an elongation of the occipital part of the cranium in zebras and donkeys, which previously have been shown to be a distinct character to separate these two taxa from the domesticated horses (for a more detailed morphological description of the skulls of each group see Table 4, Fig. 3, [38, 39]).

In contrast to the results of the cranial analysis, we did not find clear group-distinguishing shape differences in the mandible or in any of the investigated teeth. We found the highest Procrustes variance in the mandible data set, very closely followed by the cranium. All four teeth showed a lower Procrustes variance pointing towards less variability, a result also reflected in the low magnitude of disparity for landmarks belonging to the molar (MR) module, probably related to dietary constraints. Our findings on tooth shape differences are congruent with the "long-fuse" hypothesis on teeth by Seetah et al. [14], stating that "shape changes in equids have been modest […] until the development of modern breeds in recent centuries". Cucchi et al. [16], however, found a strong taxonomic pattern in the shape of the enamel folding, allowing for a more distinct taxonomic separation at the species level. These resulting differences are most likely due to the different choice of teeth ([14]: UP4; [16]: LP3 – LM2) , as suggested by one of the articles [16], different

Table 4 Description of morphological differences for domesticated and wild equids for the studied sample (for a detailed sample composition see Table 2)

Type	Domesticated equids				Wild equids		
Trait	Draft horses ($n = 37$)	Ponies including Falabella ($n = 40$)	Medium horses ($n = 38$)	Light horses ($n = 14$)	Przewalski's horses ($n = 5$)	Donkeys ($n = 33$)	Zebras ($n = 47$)
Average skull length based on studied sample (±SD)	56 (±2.5) cm	43 (±3.6) cm	52 (±4.5) cm	51 (±2.3) cm	46 (±2.1) cm	41 (±4.6) cm	48 (±4.1) cm
General skull shape	Skull breadth is half of skull length	Skull breadth is half of skull length	Elongated	Elongated	Skull breadth is half of skull length	Skull breadth is almost skull length	Very elongated in the Grevy Zebra
Frontals	Broad	Broad in some breeds	Broad in some breeds	Narrow	Narrow	Narrow	Narrow
Degree of cranial flexion (after Bennett, 1980)	High	High in smaller breeds	Medium	High	Medium	High	Low
Nasals	Convex; End at ventral end of premaxillary-maxillary suture	Concave in smallest breed, straight in larger breeds; End before ventral end of premaxillary-maxillary suture	Straight with exceptions in some breeds; End at ventral end of premaxillary-maxillary suture	Concave; End at ventral end of premaxillary-maxillary suture	Straight or slightly concave; End at ventral end of premaxillary-maxillary suture	Straight or slightly concave; End at ventral end of premaxillary-maxillary suture	Straight or slightly concave; End at ventral end of premaxillary-maxillary suture
Angle between premaxilla and maxilla	Obtuse	Obtuse, almost straight	Straight	Straight	Right to obtuse	Obtuse to straight	Obtuse to straight
Facial crest	Prominent	Prominent	Prominent	Less prominent	Prominent	Prominent	Prominent
Zygomatic, temporal and postorbital bars	Very broad, Horizontal to skull midline	Broad to normal; Horizontal to skull midline	Thin, with the temporal being broader; Horizontal to skull midline	Very thin; Horizontal to skull midline	Normal; Angled to skull midline	Normal; Angled to skull midline	Very broad; Angled to skull midline
Orbit shape	Roundish with a more squared part at the dorso-posterior edge	Almost round	Egg-shaped	Oval	Oval	Oval	Round to egg-shaped
Zygomatic process of the temporal bone	Flat	Flat	Curved	Curved	Flat	Flat	Flat
Length of the lambdoidal crest	Does not overlap the condyles in lateral view	Does not overlap the condyles in lateral view	Does not overlap the condyles in lateral view	Does not overlap the condyles in lateral view	Overlaps the condyles in lateral view	Overlaps the condyles in lateral view	Overlaps the condyles in lateral view
Cranial vault	Flat	Rounded in smaller breeds	Flat	Flat	Flat	Rounded	Flat

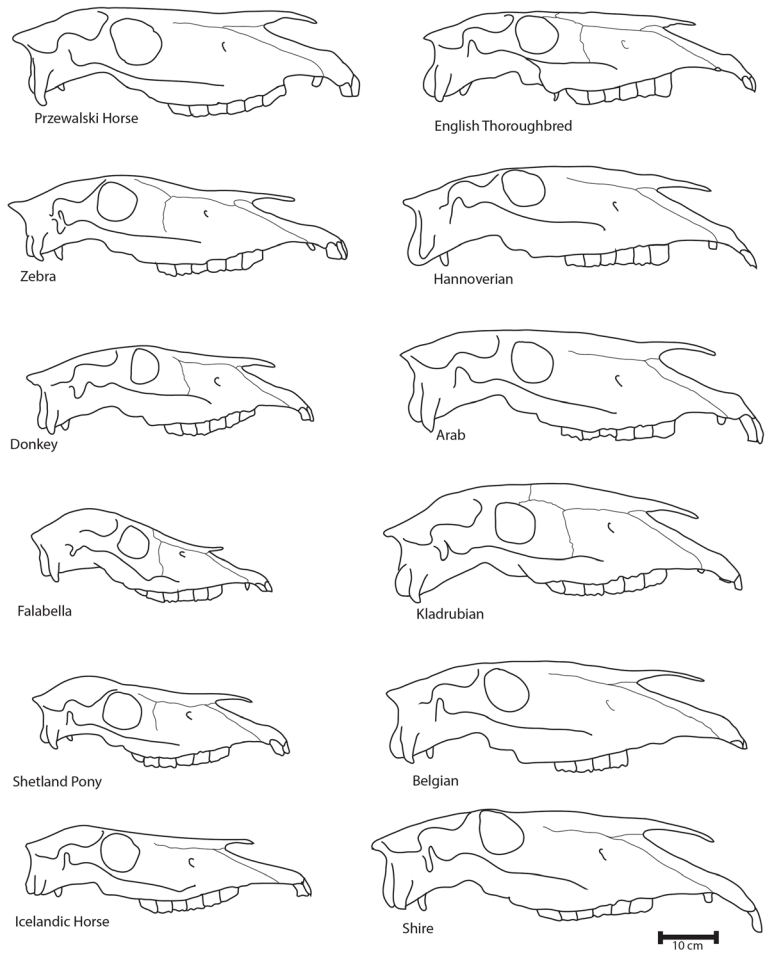

Fig. 3 Examples for skull shapes from lateral view of different domesticated (Draft: Shire, Belgian; Light horse: Arab; Medium horses: Hannoverian, Kladrubian, Engl. Thoroughbred; Ponies: Falabella, Shetland, Icelandic) and wild equids (Przewalski's horse, Zebra, Donkey)

wear stages, and/or the different methods used (landmarks vs. semilandmarks).

Modular patterning of the cranium is well-supported by empirical evidence, representing shared development and functional associations between parts of the cranium, resulting in their parcellation into semi-autonomous units. Consistent with patterns recovered for other placental mammals [20], our results indicate that trait variation in the equid cranium is best supported by a six-module hypothesis. These six modules reflect functional groups: the anterior oral-nasal (AON) and molar (MR) modules represents the primary masticatory apparatus, the zygomatic-pterygoid (ZP) module includes the region of attachment for masticatory muscles, the orbit (ORB) module contains the visual sensory organs, the cranial vault (CV) supports and protects the brain, and the cranial base (CB) supports the braincase and is the point of attachment between the skull and axial skeleton. Previous analyses of module disparity and integration for these six modules in a sample of carnivorans provided some support for highly integrated modules showing low disparity, particularly the basicranium (CB), and weakly integrated modules showing high disparity (ORB and ZP) [20, 28]. Our results are broadly consistent with this trend (Fig. 2), which is suggestive of strong integration acting to limit trait variation or the direction of response to selection. The ZP module in horses is also found to be weakly integrated and showing high disparity. Among the carnivoran sample, the molar region showed an unusual pattern of high disparity and high levels of integration [28], in our sample the molar module is also recovered as the most highly integrated module but displays the lowest levels of disparity for both wild and domesticated forms. The discrepancy between these results is likely explained by the diversity of dietary habits represented by the carnivoran sample (e.g. hypercarnivores, insectivores, frugivores) in that earlier study, which resulted in a high disparity among the landmarks captured in the molar module.

Selection acting on shared developmental and functional processes can result in an uncoupling of trait associations at different levels [40–43], providing evidence for complex interactions between modularity and selection. Following, it might therefore be expected that domestication events, as examples of selective breeding regimes, could alter patterns of modularity and integration, and these alterations may differ among breeds, acknowledging that the features targeted for selection (e.g. gait, conformation) are likely to differ for some breeds. There exist few empirical tests of this hypothesis and results on dogs are inconclusive, with reports that patterns of integration have remained stable despite the morphological diversification associated with domestication [9, 44], but also that high module disparity is associated with greater cranial shape variation in dogs compared to wolves [45]. In contrast to Parr et al. [45] our results show highly similar magnitudes of module disparity among wild and domesticated forms, and instead lower magnitudes of module integration are recovered in domesticated horses compared to their wild relatives. Variability in integration magnitude, as recovered here, rather than patterning has been proposed to underlie cranial diversity in mammals [31, 32], such that general conservatism in patterning across mammals may be explained as a product of stabilizing selection on basic developmental processes whereas directional selection could act by altering magnitudes of integration. A recent study conducted simulations to test the role of integration in generating morphological disparity and noted that integration may not affect disparity in morphospace in the way that it is usually measured (as a volume of occupied morphospace or as a measure of dissimilarity), making the relationship between morphospace occupation and modularity results potentially difficult to interpret [46]. That study did not compare shape variation and its partitioning into modules, however our PCA plots indicate that the main axes of shape variation in the equid sample are spread across landmarks located in at least three modules (CV, AON and ZP). Similarly, Parr et al. [45] found shape variance in wild and domesticated dogs to be spread across modules with different magnitudes of integration. It has been suggested that modularity may repartition variance along new directions in morphospace, thereby exploring a greater volume, however the so-far limited empirical evidence appears to raise the question of the extent to which those new directions may be aligned with the axes recovered by eigen-decomposition of shape variables into mathematically orthogonal axes, as happens in ordination techniques such as PCA.

Methods

A total of 216 crania, 224 mandibles, 198 upper and 141 lower second premolars (U2P and L2P respectively), and 192 upper and 144 lower third molars (U3M and L3M respectively) were analysed (Table 2).

We examined specimens from the following collections: Museum für Naturkunde Berlin (MfN Berlin, Germany), Institut für Haustierkunde (Christian-Albrechts-Universität of Kiel, Germany), Museum für Haustierkunde "Julius Kühn" (University of Halle, Germany), Naturhistorisches Museum Wien (NHW Vienna, Austria), and Museo de la Plata (MLP La Plata, Argentina). The dataset includes all recent species of the genus *Equus* [37]. Due to inconsistent species assignment within zebras and donkeys across museums, we analysed all zebra (cranium $n = 47$; mandible $n = 48$) and donkey (cranium $n = 31$; mandible $n = 33$) species as one group, respectively. We further included five crania and two mandibles of Przewalski's horses. The largest number of specimens in our data set belongs to the domesticated horses (cranium $n = 133$; mandible $n = 141$) including the following breeds: Ancient Breed (Roman period), Anglo-Norman, Arab, Birkenfelder, Belgian Draft, Bosnian Pony, Clydesdale, Exmoor Pony, Falabella, Galician Farm Horse, Grisons (Graubündner), German Riding Pony, Hannoverian, Hackney, Holstein, Hungarian, Huzule, Icelandic Horse, Indian Pony, Kladrubian, Konik, Kosarian, Lipizzan, Mongolian, Norik, Oldenburgian, Pinzgau, Polish Farm Horse, Scottish Pony, Seneca Sarajevo, Shetland Pony, Shire, Styrian, Suffolk, English Thoroughbred, Togo Pony, Trakehner, and Welsh (Table 2).

Analyses of cranial, mandibular and teeth size and shape were performed using landmark-based geometric morphometric (GMM) approaches. The crania and mandibles were measured in three-dimension (3D) using a MicroScribe ® MLX6 (Revware, Inc., Raleigh, North Carolina, USA), while the teeth were measured in two-dimension (2D) using high resolution photographs. A total of 62 type I and type II landmarks [47] were collected on the cranium (Table 5, Additional file 3). The dorsal and ventral sides of the crania (Fig. 4) were measured separately and were subsequently combined using three reference landmarks (numbered 1, 2, and 33, Table 5). For the mandible 24 type II landmark coordinates were measured (Table 5, Fig. 4, Additional file 3).

Phenotypic variation of the four studied teeth was assessed using 9 to 12 two-dimensional landmarks (Type II) (Table 5, Fig. 4, Additional file 4, 5, 6 and 7) following Seetah et al. [48] for the upper teeth that was adapted for the lower teeth. The landmark coordinates were collected on high resolution photographs using TPSDig2 [49]. The photographs were all taken in a standardized manner using a Canon Eos 600d with a Canon EF 24-105 mm f/4 L S USM lens from lateral and dorsal view with a scale bar for size reference.

Geometric morphometric analyses

To eliminate the effects of size, orientation, and scaling we performed General Procrustes Analysis (GPA, [50]),

Table 5 Description of the landmarks, including position and type, collected on each cranium, mandible, and tooth; Type I: discrete juxtapositions of tissue types and Type II: maxima of curvature or other local morphogenetic processes [37]

	Position	Type
Cranium		
1–2	Posterior tip of the upper third incisor	II
3–4	Posterior most point of the nasal-premaxilla suture	I
5–6	Premaxillary-maxillary-nasal suture	I
7–8	Dorsoposterior tip of the infraorbital foramen	II
9–10	Anterior tip of the facial crest	II
11	Nasion, nasal-frontal suture, midline	I
12–13	Junction of the lacrimal, maxilla, and nasal sutures	I
14–15	Zygo-lacrimal suture on the orbital margin	I
16–17	Lacrimal-frontal suture on the orbital margin	I
18–19	Supraorbital foramen	II
20–21	Anterior tip of the zygo-temporal suture	I
22–23	Posterior tip of the zygo-temporal suture	I
24–25	Dorsal tip of the frontal-temporal suture	I
26–27	Ventroposterior tip of the zygomatic process	II
28–29	Dorsalmost point of the vertically orientated posterior margin of the zygomatic process	II
30–31	Ventrolateralmost point of squamous part of temporal bone	II
32	Anterior tip of the occipital triangle	I
33	Posterior tip of the nuchal crest	II
34–35	Dorsolateral tip of the nuchal crest	II
36	Dorsalmost point on the margin of the foramen magnum	II
37	Point between first incisors from ventral side	II
38–39	Posteriormost tip of the premaxillary-maxillary suture, ventral	I
40–42	Anterior tip of the second premolar	II
41–43	Posterior tip of the third molar	II
44	Posteriormost point of the incisive canal	II
45	Posterior tip of the palatine process of the incisive bone	I
46	Posterior tip of the palatine-palatine suture	I
47–48	Distal tip of the pterygoid hamulus	II
49–51	Anterior tip of the caudal alar foramen	II
50	Posterior tip of the vomer on the midline	II
52–53	Medial tip of the mandibular fossa	II
54–55	Canal for hypoglossal nerve	II
56–57	Fossa medial of the paracondylar process	II
58–59	Distal tip of the paracondylar process	II
60	Ventral tip of the foramen magnum	II
61–62	Posteriormost tip of the occipital condyle	II
Mandible		
U1	Posterior point between first incisors	II
U2–U3	Posterior tip of the third lower incisor	II
U4–U5	Posterior tip of the canine	II
U6	Posterior tip of the mandible on the midline	II
U7–U8	Anterior tip of the second premolar	II

Table 5 Description of the landmarks, including position and type, collected on each cranium, mandible, and tooth; Type I: discrete juxtapositions of tissue types and Type II: maxima of curvature or other local morphogenetic processes [37] *(Continued)*

	Position	Type
U9–U10	Posterior tip of the third molar	II
U11–U12	Junction of the bases of the coronoid and condylar processes	II
U13–U14	Lateral tip of the condylar process	II
U15–U16	Medial tip of the condylar process	II
U17–U18	Posterior tip of the mandibular mental foramen	II
U19–U20	Vascular notch of the mandible	II
U21–U22	Maximum curvature of the angle of the mandible right behind the vascular notch	II
U23–U24	Maximum curvature of the angle of the mandible	II
Cranium 2P		
1	Maximum curvature of the metastyle	II
2	Maximum curvature of the mesostyle, distal side	II
3	Maximum curvature of the mesostyle, medial side	II
4	Maximum curvature of the anterior accessory rib	II
5	Maximum curvature of the parastyle	II
6	Maximum curvature of the protocone, mesial/labial side	II
7	Maximum curvature of the protocone, buccal side	II
8	Maximum curvature of the protocone, distal/labial side	II
9	Maximum curvature of the hypocone	II
Cranium 3 M		
1	Maximum curvature of the metastyle	II
2	Maximum curvature of the mesostyle	II
3	Maximum curvature of the parastyle	II
4	Maximum curvature of the preprotoconal groove	II
5	Maximum curvature of the protocone, mesial side	II
6	Maximum curvature of the protocone, distal side	II
7	Maximum curvature of the post protoconal valley, buccal side	II
8	Maximum curvature of the post protoconal valley, labial side	II
9	Maximum curvature of the hypocone, buccal side	II
10	Maximum curvature of the hypocone, labial side	II
Mandible 2P		
1	Maximum curvature of the protoconid, anterior/ lingual side	II
2	Maximum curvature of the preflexid, anterior side	II
3	Maximum curvature of the preflexid, posterior side	II
4	Maximum curvature of the metaconid, anterior side	II
5	Maximum curvature of the metastylid, posterior side	II
6	Maximum curvature of the postflexid, anterior side	II
7	Maximum curvature of the postflexid, posterior side	II
8	Maximum curvature of the hypoconulid, posterior side	II
9	Maximum curvature of the hypoconid, anterior/ buccal side	II
10	Maximum curvature of the ectoflexid, lingual side	II
11	Maximum curvature of the protoconid, posterior/ buccal side	II
12	Maximum curvature of the protoconid, anterior/ buccal side	II

Table 5 Description of the landmarks, including position and type, collected on each cranium, mandible, and tooth; Type I: discrete juxtapositions of tissue types and Type II: maxima of curvature or other local morphogenetic processes [37] *(Continued)*

	Position	Type
Mandible 3 M		
1	Maximum curvature of the hypoconulid, posterior side	II
2	Maximum curvature of the entoconid, anterior side	II
3	Maximum curvature of the entoflexid, posterior side	II
4	Maximum curvature of the entoflexid, anterior side	II
5	Maximum curvature of the metastylid, posterior side	II
6	Maximum curvature of the metaconid, anterior side	II
7	Maximum curvature of the metaflexid, posterior side	II
8	Maximum curvature of the protoconid, anterior side	II
9	Maximum curvature of the protoconid, posterior side	II
10	Maximum curvature of the ectoflexid, lingual side	II
11	Maximum curvature of the hypoconid, anterior/ buccal side	II
12	Maximum curvature between the hypoconid and hypoconulid, lingual side	II

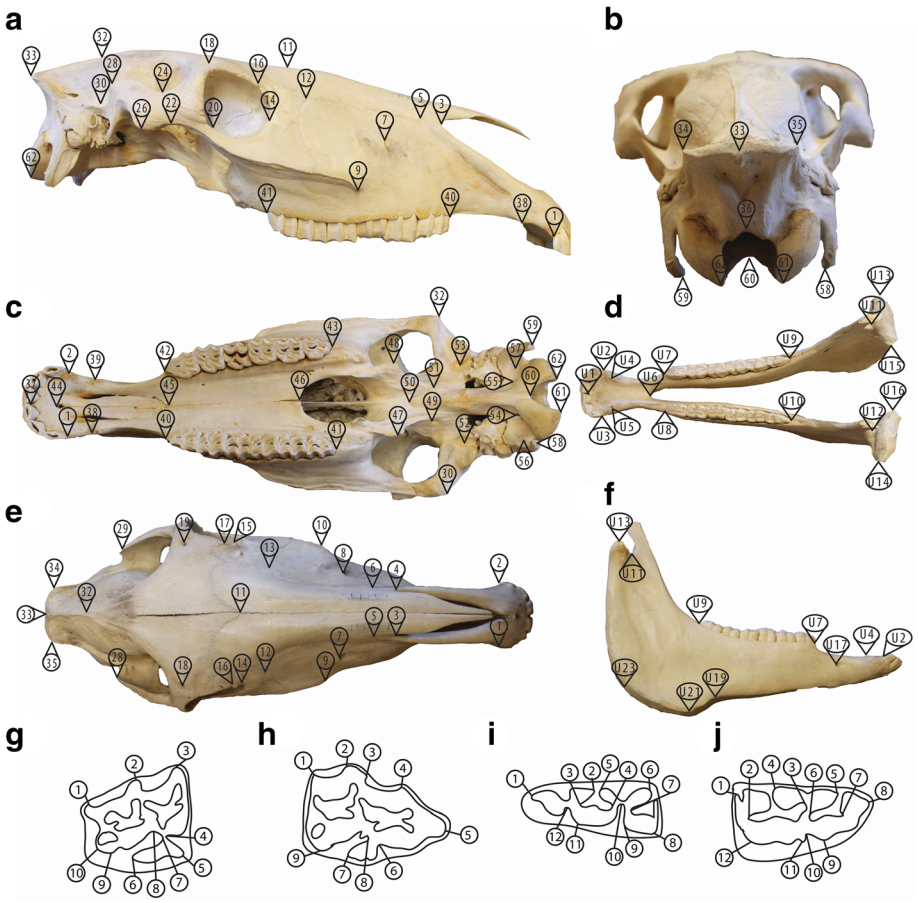

Fig. 4 Landmarks on the **a** lateral **b** ventral **c** dorsal **d** posterior side of the skull and the **e** dorsal **f** lateral side of the mandible of a domesticated horse (for a detailed description of the landmarks see Table 3); Landmarks on the **g** upper 3rd molar **h** upper 2nd premolar **i** lower 3rd molar **j** lower 2nd premolar of a zebra (specimen MfN 70,299) in occlusal view (for a detailed description of the landmarks see Table 3)

which translates all specimens' coordinates so their centroid coincides, scales them to unit centroid size, and rotates them to minimize squared summed distances between matching landmarks. With the cranium and the mandible being symmetric objects, only the symmetric component of shape was analysed in subsequent procedures [51]. The Procrustes scores retained from the GPAs for each skeletal feature were subjected to Principal Component Analyses (PCA). Differences in shape among the four different equid taxa were explored using Procrustes analysis of variance (ANOVA) [52] with shape (PC scores) as the dependent and group (horses [H], Przewalski's horses [P], zebras [Z], and donkeys [D]) as the independent variable. Canonical Variance Analyses (CVA) was then performed to identify the shape features which best characterize the different groups. Due to the high dimensionality of the datasets, a dimensionality reduction was performed prior to the ANOVA and CVA analyses using the mevolCVP function in R [12]. The mevolCVP function helps to identify the appropriate number of dimensions (first PC scores) which maximize the cross-validated percentage in the subsequent analyses using leave-one-out cross-validated linear discriminant analyses (LDA) (for a more detailed explanation see [53]). We then only used the predetermined number (N) of first PC scores to test for differences in shape among the defined groups using Procrustes ANOVA. If the Procrustes ANOVA showed significant results, we performed a Canonical Variance Analysis (CVA) to identify the shape features which best characterize the different groups. Because sample sizes of Przewalski's horses were relatively small, CVA analyses were performed with and without them. When they were excluded, predictive CVAs were used to assess the proximity of these specimens with the three remaining groups (identification were based on resampled designs [15]).

We determined distances among the groups by calculating squared Mahalanobis distances (D^2), which represents the distance of one group mean to another group mean in standard deviations.

Further, we analysed the morphological disparity (as Procrustes variance, [52]) which is the occupied space of all specimens together in multidimensional shape space [54]. First, we calculated the grand mean in unit Procrustes variances. Then we inferred and compared Foote's partial disparity (PD) [54, 55] to the grand mean. PD was calculated for each group (H, P, D, Z) separately, and for wild equids (D, Z, P) and domesticated horses (H). To do so, the residuals from the regression of shape across all specimens were used and the squared residual lengths were summed over either group mean. The resulting group wise Procrustes variances were multiplied by the number of samples per group divided by total sample size minus one. We then calculated the contribution in percent of each group to the overall disparity.

Analyses were conducted using R [56] in RStudio (v.1.0.136) and related R packages [52, 57, 58] (R script is available upon request). The analyses were computed separately for the cranium, the mandible, and each of the four teeth.

Modularity analyses

Cranial landmarks for the total sample (wild equids and domesticated horses) were tested for modular structure using 17 models. Wild equids and domesticated horses were pooled because modular patterning has been demonstrated to be stable across placental mammals [20, 31, 32]. With the exception of the simplest model (= no modularity), cranial landmarks were subdivided into modules following a priori hypotheses for modular patterning. These were: 1) Tissue origin hypothesis (neural crest vs paraxial mesoderm derived elements [33]), 2) adult module hypothesis [20], 3) Cheverud's functional module hypothesis [59], and 4) horse-specific hypothesis [35, 60] (see Additional file 8: Table S3). Hypotheses #1–3 have previously been tested on a macroevolutionary scale in mammals whereas hypothesis #4 tests the face and neurocranium as two separate units based on previously recovered growth pattern differences of the face relative to the neurocranium in horse evolution [35, 36, 60]. For each of these competing hypotheses (#1–4) we compared the fit of our data to different model structures, allowing for variation in correlation within and between modules. As such, each hypothesis was evaluated for four variants (a-d), these were a) same within-module integration and same between-module integration, b) same within-module integration and separate between-module integration, c) separate within-module integration and same between-module integration, and d) separate within-module integration and separate between-module integration (see Additional file 8: Table S3). The fit of the 17 models (4 hypotheses × 4 variants [a-d, above] plus 'no modularity' hypothesis) was evaluated using the EMMLi package version 0.0.3 [22] in R, using a coordinate (Procrustes aligned) correlation matrix based on absolute values of correlations as input. EMMLi is a maximum likelihood approach that allows for the direct comparison of models of mixed complexity, and outputs a corrected Akaike Information Criterion (AICc) value and an AICc difference (dAICc), which can be used to assess the fit of the model to the data [22].

The best supported model of modularity (lowest AICc and smallest dAICc) recovered from the EMMLi analysis was chosen for further calculations of module disparity and integration and comparisons between wild and domesticated forms. The cranial landmark matrix was

subdivided into matrices for domesticated horses and wild equids. The matrices for domesticated horses and wild equids were each further subdivided into module-specific landmark sets (e.g. orbit module domesticated horses, orbit module wild equids) and subject to GPA. For each module, disparity of the landmarks within that module was defined as maximum Procrustes distance following previous studies (e.g. [45]), and was calculated using Procrustes distances between the mean shape landmark configuration and the landmark configuration of each specimen. Disparity calculations were performed using the Evomorph package version 0.9 [61] in R. For each module, integration of the landmarks within that module was calculated using relative eigenvalue standard deviation (i.e. eigenvalue dispersion), following calculations detailed in [62]. This measure assesses the variance of extracted eigenvalues, which would be maximal when all variation in the data is found in a single dimension (i.e. complete integration) and zero when all eigenvalues are equal (i.e. no integration [30]). Therefore, large values for eigenvalue dispersion reflect strong integration between the landmarks in a module. Eigenvalue dispersion has been shown to be independent of trait number and highly correlated with the mean squared correlation coefficient [32].

Conclusion

We described and compared shape changes in various skeletal features among extant equid species using geometric morphometrics. Our results support Darwin`s hypothesis that shape variation in the skull of domesticated horses is similar to the shape variation of all wild extant equid species. Our study further shows that lower magnitudes of integration among six cranial modules are found in domesticated horses compared to their wild relatives. Future research could address the relation between integration and disparity, investigating the relation between the two during the domestication process of diverse species.

Additional files

Additional file 1: Table S1. List of cranial landmarks and their placement within the module configurations tested in this study. Four modularity hypotheses were tested, see text for further details. Modules for each hypothesis are as follows; 1. Tissue origin – neural crest (NC), paraxial mesoderm (PM); 2. Mammalian modules – anterior oral-nasal (AON), cranial base (CB), cranial vault (CV), molar (M), orbital (ORB), zygomatic pterygoid (ZP); 3. Functional modules – basicranium (B), frontal (F), masticatory (M), nasal (N), oral (O), orbital (OB); 4. Horse-specific – brain (BR), teeth (TE). (DOCX 15 kb)

Additional file 2: Table S2. Results from EMMLi analyses, showing the best (highlighted) supported model of modularity for the cranial landmark data set. Details show the model parameters (K), maximum and log-likelihood values for each tested model, as well as the corrected Akaike Information Criterion (AICc), and the difference between the AICc for a model and the overall minimum AICc (dAICc). The number of between-trait correlations considered in calculating the model likelihood for the sample is 1891, which is equal to the number of unique subdiagonal values of the matrix. Model ID values correspond to those provided in the Material and Methods text. (DOCX 15 kb)

Additional file 3: Raw data for crania and mandibles for all specimens used in this study including three-dimensional landmark data (raw coordinates) and identifier; ID_String is the individual combination including all information: Museum (A = Argentina, B = Berlin, H = Halle, K = Kiel, V = Vienna), ID (identifier used at the museum), group (H = horse, D = donkey, P = Przewalski's, Z = zebra), breed (aaa = not a domesticated horse, ahb = Ancient Breed (Roman period), ano = Anglo-Norman, arb = Arab, bif = Birkenfelder, blg = Belgian Draft, bos = Bosnian Pony, cds = Clydesdale, exm = Exmoor Pony, fab = Falabella, gbh = Galician Farm Horse, grb = Grisons (Graubündner), grp = German Riding Pony, han = Hannoverian, hny = Hackney, hol = Holstein, hun = Hungarian, huz = Huzule, ice = Icelandic Horse, ind = Indian Pony, kdr = Kladrubian, kon = Konik, kos = Kosarian, lpz = Lipizzan, mon = Mongolian, nor = Norik, odb = Oldenburgian, piz = Pinzgau, pll = Polish Farm Horse, scp = Scottish Pony, ses = Seneca Sarajevo, she = Shetland Pony, shi = Shire, stm = Styrian, suf = Suffolk, tbh = English Thoroughbred, tog = Togo Pony, trk = Trakehner, and wel = Welsh), and morphotype (A = not a domesticated horse, W = medium horse, F = Light horse, C = Draft horse, P = Pony). (XLSX 13 kb)

Additional file 4: TpsDig output for 2) upper third molar, 3) upper second premolar, 4) lower third molar, and 5) lower second premolar for all specimens used in this study including two-dimensional landmark data (raw coordinates) and identifier (image name); for details see Additional file 1. (TXT 13 kb)

Additional file 5: TpsDig output for 2) upper third molar, 3) upper second premolar, 4) lower third molar, and 5) lower second premolar for all specimens used in this study including two-dimensional landmark data (raw coordinates) and identifier (image name); for details see Additional file 1. (TXT 12 kb)

Additional file 6: TpsDig output for 2) upper third molar, 3) upper second premolar, 4) lower third molar, and 5) lower second premolar for all specimens used in this study including two-dimensional landmark data (raw coordinates) and identifier (image name); for details see Additional file 1. (TXT 12 kb)

Additional file 7: TpsDig output for 2) upper third molar, 3) upper second premolar, 4) lower third molar, and 5) lower second premolar for all specimens used in this study including two-dimensional landmark data (raw coordinates) and identifier (image name); for details see Additional file 1. (TXT 12 kb)

Additional file 8: Table S3. Module disparity and integration values calculated separately for domesticated and wild horses. Modules are, anterior oral-nasal (AON), cranial base (CB), cranial vault (CV), molar (MR), orbital (ORB), and zygomatic-pterygoid (ZP), as recovered by Goswami (2006) (see Materials and Methods for further details). (DOCX 14 kb)

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Availability of data and materials

The datasets used and/or analysed during the current study are available in the supplementary information to this article.

Authors' contributions
LH collected the data and performed and interpreted the geometric morphometric analyses. LABW performed and interpreted the modularity analyses. AE provided the R functions and pipeline used in the geometric morphometric analyses. MS performed and interpreted the Foote's partial disparity analyses and supported LH during the geometric morphometric analyses. MRS-V conceived and supervised the entire study. All authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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Chapter III - Supplement

Supplementary Table S1: Results from EMMLi analyses, showing the best (highlighted) supported model of modularity for the cranial landmark data set. Details show the model parameters (K), maximum and log-likelihood values for each tested model, as well as the corrected Akaike Information Criterion (AICc), and the difference between the AICc for a model and the overall minimum AICc (dAICc). The number of between-trait correlations considered in calculating the model likelihood for the sample is 1891, which is equal to the number of unique subdiagonal values of the matrix. Model ID values correspond to those provided in the Material and Methods text.

Model ID	Description	Max. Likelihood	K	AICc	dAICc	Model Likelihood	Posterior probability
0	No.modules.default	-1803.56	2	3611.131	4580.472	0	0
1a	Tissue.same.Mod + same.between	-1737.9	4	3483.825	4453.167	0	0
1b	Tissue.same.Mod + sep.between	-1737.9	4	3483.825	4453.167	0	0
1c	Tissue.sep.Mod + same.between	-1056.95	5	2123.929	3093.27	0	0
1d	Tissue.sep.Mod + sep.between	-1056.95	5	2123.929	3093.27	0	0
2a	Goswami.same.Mod + same.between	-877.315	4	1762.651	2731.993	0	0
2b	Goswami.same.Mod + sep.between	-313.031	18	662.4267	1631.768	0	0
2c	*Goswami.sep.Mod + same.between*	*-56.3181*	*9*	*130.7318*	*1100.073*	*1.32E-239*	*1.32E-239*
2d	Goswami.sep.Mod + sep.between	507.9663	23	-969.341	0	1	1
3a	Functional.same.Mod + same.between	-1085.46	4	2178.933	3148.274	0	0
3b	Functional.same.Mod + sep.between	-487.41	18	1011.185	1980.527	0	0
3c	Functional.sep.Mod + same.between	-945.554	9	1909.204	2878.545	0	0
3d	Functional.sep.Mod + sep.between	-347.508	23	741.6077	1710.949	0	0
4a	horse.same.Mod + same.between	-1218.03	4	2444.088	3413.429	0	0
4b	horse.same.Mod + sep.between	-1218.03	4	2444.088	3413.429	0	0
4c	horse.sep.Mod + same.between	-1036.53	5	2083.093	3052.434	0	0
4d	horse.sep.Mod + sep.between	-1036.53	5	2083.093	3052.434	0	0

Supplementary Table S2: Module disparity and integration values calculated separately for domesticated and wild horses. Modules are, anterior oral-nasal (AON), cranial base (CB), cranial vault (CV), molar (MR), orbital (ORB), and zygomatic-pterygoid (ZP), as recovered by Goswami (2006) (see Materials and Methods for further details).

Module	Integration (Eigenvalue dispersion)		Disparity (Procrustes distance)	
	Domesticated	Wild	Domesticated	Wild
AON	0.532187	0.59987	0.043257	0.04116
CB	0.711104	0.785558	0.026985	0.029178
CV	0.740895	0.785045	0.033325	0.030771
MR	0.807072	0.840303	0.026379	0.021098
ORB	0.72742	0.798126	0.028563	0.041428
ZP	0.554181	0.581242	0.046471	0.039242
average	0.678809	0.731690	0.034163	0.033812

Supplementary Table S3: List of cranial landmarks and their placement within the module configurations tested in this study. Four modularity hypotheses were tested, see text for further details. Modules for each hypothesis are as follows; 1. Tissue origin – neural crest (NC), paraxial mesoderm (PM); 2. Mammalian modules – anterior oral-nasal (AON), cranial base (CB), cranial vault (CV), molar (M), orbital (ORB), zygomatic pterygoid (ZP); 3. Functional modules – basicranium (B), frontal (F), masticatory (M), nasal (N), oral (O), orbital (OB); 4. Horse-specific – brain (BR), teeth (TE).

Cranial Landmark #	Modularity hypothesis			
	1. Tissue origin	2. Mammalian modules (Goswami)	3. Functional modules (Cheverud)	4. Horse-specific (Radinsky)
1	NC	AON	O	TE
2	NC	AON	O	TE
3	NC	AON	N	TE
4	NC	AON	N	TE
5	NC	AON	N	TE
6	NC	AON	N	TE
7	NC	AON	N	TE
8	NC	AON	N	TE
9	-	-	-	-
10	-	-	-	-
11	NC	ORB	N	TE
12	NC	ORB	N	TE
13	NC	ORB	N	TE
14	NC	ORB	OB	-
15	NC	ORB	OB	-
16	NC	ORB	OB	-
17	NC	ORB	OB	-
18	NC	ORB	F	-
19	NC	ORB	F	-
20	NC	ZP	OB	-
21	NC	ZP	OB	-
22	NC	ZP	M	-
23	NC	ZP	M	-
24	NC	ZP	M	-
25	NC	ZP	M	-
26	NC	ZP	M	-
27	NC	ZP	M	-
28	NC	ZP	M	-
29	NC	ZP	M	-
30	NC	ZP	M	-
31	NC	ZP	M	-
32	PM	CV	F	BR
33	PM	CV	F	BR
34	PM	CV	F	BR
35	PM	CV	F	BR
36	PM	CV	B	BR
37	NC	AON	O	TE
38	NC	AON	O	TE
39	NC	AON	O	TE
40	NC	AON	O	TE
41	NC	MR	O	TE
42	NC	AON	O	TE
43	NC	MR	O	TE
44	NC	AON	O	TE
45	NC	AON	O	TE
46	NC	MR	O	TE
47	-	-	-	-
48	-	-	-	-
49	NC	ZP	B	BR
50	NC	ZP	B	BR
51	NC	ZP	B	BR
52	NC	ZP	B	BR
53	NC	ZP	B	BR
54	PM	CB	B	BR

Supplementary Table S3 (continued)

55	PM	CB	B	BR
56	PM	CB	B	BR
57	PM	CB	B	BR
58	PM	CB	B	BR
59	PM	CB	B	BR
60	PM	CB	B	BR
61	PM	CB	B	BR
62	PM	CB	B	BR

Additional File 1: Raw data for crania and mandibles for all specimens used in this study including three-dimensional landmark data (raw coordinates) and identifier; ID_String is the individual combination including all information: Museum (A = Argentina, B = Berlin, H = Halle, K = Kiel, V = Vienna), ID (identifier used at the museum), group (H = horse, D = donkey, P = Przewalski's, Z = zebra), breed (aaa = not a domesticated horse, ahb = Ancient Breed (Roman period), ano = Anglo-Norman, arb = Arab, bif = Birkenfelder, blg = Belgian Draft, bos = Bosnian Pony, cds = Clydesdale, exm = Exmoor Pony, fab = Falabella, gbh = Galician Farm Horse, grb = Grisons (Graubündner), grp = German Riding Pony, han = Hannoverian, hny = Hackney, hol = Holstein, hun = Hungarian, huz = Huzule, ice = Icelandic Horse, ind = Indian Pony, kdr = Kladrubian, kon = Konik, kos = Kosarian, lpz = Lipizzan, mon = Mongolian, nor = Norik, odb = Oldenburgian, piz = Pinzgau, pll = Polish Farm Horse, scp = Scottish Pony, ses = Seneca Sarajevo, she = Shetland Pony, shi = Shire, stm = Styrian, suf = Suffolk, tbh = English Thoroughbred, tog = Togo Pony, trk = Trakehner, and wel = Welsh), and morphotype (A = not a domesticated horse, W = medium horse, F = Light horse, C = Draft horse, P = Pony).

Additional File 2 - 5: TpsDig output for 2) upper third molar, 3) upper second premolar, 4) lower third molar, and 5) lower second premolar for all specimens used in this study including two-dimensional landmark data (raw coordinates) and identifier (image name); for details see additional file 1.

Additional files are provided in electronic form at the end of this thesis.

Chapter IV

Are miniature horse breeds miniature horses?

A study of skull shape and growth

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Abstract

Much of the novel shape variation found in domesticated animals is due to heterochrony, allometry, and neomorphy. Some of the phenotypic changes, especially in small breeds, have been suggested to represent a form of paedomorphism. Due to their considerable size range (wither height from around 70 cm to 2 m), horses are an excellent study subject to investigate patterns of size-related shape change. We investigated whether crania of miniature horse breeds (wither height below 97 cm) can be described as paedomorphic using a dataset of 164 specimens from regular-sized horse breeds and 30 specimens of two miniature breeds, Falabella and Shetland pony. Using geometric morphometric methods, linear measurements, and multivariate analyses, we quantitatively examined the apparent juvenile cranial shape appearance of miniature horse breeds, and by looking at growth, discerned how skull shape changes postnatally. Horses show an allometric cranial growth with some cranial modules exhibiting higher variation during development than others. The two miniature breeds are not miniature forms of regular-sized breeds; they exhibit modular paedomorphic features such as enlarged orbits relative to cranial length and larger crania compared to wither height.

Keywords

Heterochrony, Falabella, pony, paedomorphism

Introduction

The domestication process and breed formation can result in a great expansion of the shape space in relation to the wild form (Darwin 1859). Much of the novel variation is due to allometry, shape changes associated with size (Herre and Röhrs 1973). Size and shape changes are associated with developmental timing, which reportedly also changes (heterochrony) in domestication (Sánchez-Villagra *et al.* 2017). Morphological novelty or neomorphism can result from domestication in any of its phases: during the initial domestication process, or in the intensive selection for particular breeds (Evin *et al.* 2017; Geiger *et al.* 2017). Horses are an excellent subject to study the latter, as the size range resulting from domestication is extensive and it has been hypothesized that disparity in cranial shape in horses is related to intraspecific allometry (Radinsky 1984; Wayne 1986). As in analogous studies in various mammal species (Drake and Klingenberg 2010; Segura *et al.* 2013; Evin *et al.* 2017; Geiger *et al.* 2017; Young *et al.* 2017), the skull can serve as a complex and appropriate marker of morphological change.

Paedomorphism, the retention of juvenile morphological or behavioral characteristics in adult age stages, has been suggested to account for some differences among horse breeds (Budiansky 1997; Goodwin *et al.* 2008). For example, small heads with medium sized bodies and long limbs are more characteristic of juvenile age stages in horses (Goodwin *et al.* 2008) than are large heads and short limbs, the latter representing a feature of juvenile stages in other species (Gould 1980). The phenotypic features which are generally considered signs for paedomorphism are differences in body proportions with a larger head and shorter limbs (Gould 1980; Heel *et al.* 2006) and differences in cranial proportions including large eyes, a more prominent and bulging cranium, and a short rostrum in combination with an enlarged braincase (Gould 1980; Wayne 1986; Evin *et al.* 2017; Tamagnini *et al.* 2017). The paedomorphism hypothesis has been stated for several domesticated species (Herre and Röhrs 1973), such as dogs (Wayne 1986; Morey 1992; Goodwin *et al.* 1997), pigs (Hilzheimer 1926), and sheep (Geist 1971).

A recent and broad taxonomic study of postnatal cranium growth in mammals hypothesized a lack of universal pattern of developmental transformation, such as paedomorphism, in the first phase of domestication (Sánchez-Villagra *et al.* 2017). With available methods, such as landmark-based geometric morphometrics, that allow for the comparison of complex shapes (Lawing and Polly 2010), the standing hypothesis, whether paedomorphism occurs in domesticated species or not, can be empirically tested. A comprehensive study on dogs recently showed that neomorphisms appear prenatally (Geiger *et al.* 2017) showing that paedomorphism is not the sole cause for novelties in cranial shape. A second study on pigs also found that significant cranial shape differences are already evident at birth (Evin *et al.* 2017). The initial phase of domestication in horses cannot be studied in this regard, as the wild form is extinct (Bowling and Ruvinsky 2000) and there are no growth series of it available in museum collections. We investigate thus, to our knowledge for the first time in domestication research, the topic of paedomorphism within domesticates only, using three-dimensional geometric morphometrics. We compare crania of two miniature horse breeds (Falabella and Shetland) to juvenile and adult crania of 37

regular-sized horse breeds (here defined as wither height of more than 130 cm), as the miniature forms are derived from selection on other domesticated forms (Hendricks 2007).

The smallest horse breed, the Falabella, originates from Argentina. The breed was first mentioned in the middle of the 19th century when very small individuals of Criollo horses were encountered in the Argentinian Pampa (Hendricks 2007). After obtaining a few individuals and starting a breeding program with miniature horses, the name giver of the breed, Juan Falabella, added small individuals of English Thoroughbred, Criollo, and Shetland pony to achieve a harmonious conformation with a wither height lower than 33 inches (84 cm). The Shetland pony, which was strongly interbred with the Falabella due to its small wither height (max. 106 cm), originates from the Shetland Islands, Scotland. It is among the oldest known horse breeds and was mostly bred locally on the islands for croft works. When an act of British Parliament, however, prohibited child labour in the coalmines in 1847, the demand for these small robust ponies, as a replacement, increased drastically. Over the last century, numerous individuals have been exported, mainly being used for driving or as a first mount for children (Hendricks 2007).

The initial visual assessment of adult specimens of Falabella and Shetland ponies showed a very distinct and more juvenile appearing cranial shape, e.g. the very roundish anterior dorsal part of the braincase, compared to specimens from regular sized breeds (Supplementary Figure 1). We use geometric morphometric methods, linear measurements, and multivariate analyses to examine quantitatively the apparent juvenile cranial shape appearance of miniature horse breeds, and by examining growth, discern how skull shape changes postnatally. First, we characterize and describe the skeletal shape difference in the cranium by visual assessment in comparison to regular sized breeds. Second, we investigate the ontogenetic shape space at all age stages of regular sized horse breeds, to create a baseline for comparison, and determine the position of miniature breeds therein. Following our main hypothesis, we expect the Falabella and adult Shetland pony specimens to cluster with young age classes of regular sized breeds.

Third, we investigate how modularity patterns characterize domesticated forms, which result from rapid evolution through artificial selection by using horses as an example. Morphological changes can occur independently in a modular fashion (Gilmour *et al.* 1993). It has been suggested the decrease of integration can increase variation (Wagner and Altenberg 1996). However, empirical data in different studies on mammals show that patterns of integration remain stable across macroevolutionary scales (Goswami 2006; Porto *et al.* 2009; Goswami *et al.* 2014). We investigate in five out of six cranial modules separately (Goswami 2006) if some individual cranial modules show more ontogenetic differences than others across all breeds and if the adult cranial modules of miniature breeds cluster with the younger age classes. Fourth, we analyze three features typically associated with paedomorphism: increased orbit size, rostral shortening, and enlarged braincase, and changed ratio of total cranial size to body size (Gould 1980; Cardini and Polly 2013).

Material and Methods

A total of 194 juvenile and adult crania were analyzed (Supplement Table 1). We

examined specimens from the following collections: Museum für Naturkunde Berlin (MfN Berlin, Germany), Institut für Haustierkunde (IfH, Christian-Albrechts-Universität of Kiel, Germany), Museum für Haustierkunde „Julius Kühn“ (University of Halle, Germany), Naturhistorisches Museum Wien (NHW Vienna, Austria), and Museo de la Plata (MLP La Plata, Argentina). The dataset includes 39 horse breeds ranging from the smallest (Falabella) to the largest breed (Shire): Ancient Breed, Anglo-Norman, Arab, Birkenfelder, Belgian Draft, Bosnian pony, Clydesdale, Exmoor pony, Falabella, Galician Farm Horse, Grisons (Graubündner), German Riding pony, Hannoverian, Hackney, Holstein, Hungarian, Huzule, Icelandic Horse, Indian pony, Kladrubian, Konik, Kosarian, Lipizzan, Mongolian, Nonius, Norik, Oldenburgian, Pinzgau, Polish Farm Horse, Scottish pony, Seneca Sarajevo, Shetland pony, Shire, Styrian, Suffolk, English Thoroughbred, Togo pony, Trakehner, and Welsh (Supplement Table 1).

Prior to analyses, each specimen was categorized into an age class from 0 to 6 using an identification key for dental eruption (Habermehl 1975) with: 0 – dental eruption of neonates (only deciduous premolars present, n=8), 1 – eruption of the first pair of deciduous incisors (n=15), 2 – eruption of the second pair of deciduous incisors (n=2), 3 – eruption of the third pair of deciduous incisors (n=10), 4 – eruption of the first molar (n=16), 5 – eruption of the second molar (n=14), and 6 – eruption of the third molar (n=129, Supplement Table 1). Weaning occurs around the end of age class 2 and the beginning of age class 3 (at around six months, Erber *et al.* 2012), while sexual maturity is reached at the beginning of age class 4 (at around one year, Kilborn *et al.* 2002) and skeletal maturity is reached in age class 5 (at around 2 years, Smith *et al.* 1999). We denote age classes 0-2 as juveniles, 3-4 as adolescent, and 5-6 as adult and these terms will be used accordingly throughout the manuscript.

Cranial shapes were analysed using landmark-based geometric morphometric (GMM) approaches. The crania were measured in three-dimension (3D) using a MicroScribe ® MLX6 (Revware, Inc., Raleigh, North Carolina, USA) and a total of 60 type I and type II landmarks (Rohlf and Bookstein 1990, Supplementary Table 2, Supplementary Figure 2) were collected. The dorsal and ventral sides of the crania were measured separately and were subsequently combined using three reference landmarks (numbered 1, 2, and 33, Supplementary Figure 2).

All subsequent analyses were conducted using R v.3.0.5 (Team 2017) in RStudio (v.1.0.136) and related R packages (Baylac 2012; Adams *et al.* 2017; Schlager 2017). R script is available upon request.

Geometric morphometric analyses

General Procrustes Analysis (GPA, Rohlf and Slice 1990) was performed to eliminate the effects of size, orientation, and scaling. GPA translates, rotates, and scales all specimens’ coordinates so their centroids coincide and are scaled to unit centroid size, and the squared summed distances between matching landmarks are minimized. All subsequent analyses were computed separately for the whole cranium and for five cranial modules (Goswami 2006; Porto *et al.* 2009), which have been confirmed for horse crania in a previous study (Heck *et al.* in review). The molar module was excluded, since no landmarks were available in this region of the cranium. Due

to its bilateral symmetry, only the symmetric component of the cranium was used in the subsequent analyses (Kolamunnage and Kent 2003). Principal component analyses (PCA) were performed using the Procrustes scores retained from the GPA. Given the high dimensionality of the datasets, a dimensionality reduction was performed prior to the MANOVA and CVA analyses using the mevolCVP function in R (Evin *et al.* 2013). The mevolCVP function helps to identify the appropriate number of dimensions (Principal component scores) which maximize the cross-validated percentage in the subsequent analyses using leave-one-out cross-validated linear discriminant analyses (LDA) (for a more detailed explanation see Price (2016)). The shape differences among the seven age classes were analysed using one-way multivariate analysis of variance (MANOVA), with Principal Component scores as the dependent and age class (0-6) as the independent variable using only the predetermined number (N) of PC scores. If the MANOVA showed significant results, a Canonical Variance Analysis (CVA) was performed to identify the shape features which best characterize the different age classes. To determine the distances of one group mean to another group mean in standard deviations, squared Mahalanobis distances (D^2) were calculated.

Further, the morphological disparity (as Procrustes variance, (Adams *et al.* 2017)) was analysed, which is the occupied space of all specimens together in multidimensional shape space (Zelditch *et al.* 2012) to further quantitively evaluate occupation in addition to the descriptive evaluation of PC1-PC2 shape space. First, the grand mean in unit Procrustes variances was calculated. Then we inferred and compared Foote's partial disparity (PD) (Foote 1993; Zelditch *et al.* 2012) to the grand mean. PD was calculated for each age class (0-6) separately. To do so, the residuals from the regression of shape across all specimens were used and the squared residual lengths were summed over either group mean. The resulting group wise Procrustes variances were multiplied by the number of samples per group divided by total sample size minus one. We then calculated the contribution in percent of each group to the overall disparity.

Testing common features of paedomorphism

To test whether miniature breeds exhibit larger orbits relative to their cranial lengths compared to regular breeds we calculated the ratio of orbit diameter (LM 15 – 17, Supplementary Table 2, Supplementary Figure 2) and total cranial length (LM 37 – 58, Supplementary Table 2, Supplementary Figure 2). Secondly, we tested for rostral shortening measuring the length of and the angle between palate (LM 37 – 44, Supplementary Table 2, Supplementary Figure 2) and basicranium (LM 49 – 58, Supplementary Table 2, Supplementary Figure 2). The angle is expected to become smaller the larger the braincase and the shorter the palate becomes (Geiger and Haussman 2016). Further, we inspected the relationship of adult cranial length (n = 128) and average breed wither height, which we collected from the literature (breeding guidelines for each breed, Supplementary Table 3). We calculated the predicted adult cranial length of the miniature breeds as derived from linear regression of adult cranial lengths from normal sized breeds (Verzani 2014) and compared it to their actual cranial lengths. The adult cranial length to wither height ratio in relation to breed is used to investigate a

possible minimal limit in cranial length in the investigated breeds. Measurements were taken from two-dimensional lateral representations of non-Procrustes aligned landmark configurations from each specimen derived from the original three-dimensional dataset. The measurements were taken using TpsDig v2.10 (Rohlf 2013).

Results

Description of cranial shape of miniature and regular-sized horse breeds

The average shape for each age class, as well as that of the adult stage of the two miniature breeds are depicted in Figure 1 and a description of different age classes (0, 3, 6) and the adult crania of the Falabella and Shetland pony are presented in Table 1. The juvenile age classes of horses are characterised by a very broad and short cranium, with a bulging anterior-dorsal part of the braincase (Figure 1, Supplementary Figure 1). During growth, the cranium elongates (elongation more pronounced in the rostrum than in the anterior part of the braincase) and the anterior-dorsal part of the cranium is more flattened. The length of the orbit decrease relative to the length of the cranium. Pictures of the Falabella cranium and an ontogenetic series of Shetland pony crania and of regular sized breed (Welsh) crania can be found in Supplementary Figure 1.

Examination of cranial shape changes related to ontogenetic development across all breeds and the position of miniature horse breeds

PCA based on all landmarks (Figure 2 A) reveals that the ontogenetic stages 0–6 separate in PC1-PC2 space along PC1 from adult (PC1 negative) to juvenile age stages (PC1 positive). The first PC accounts for 47.3% of the total shape variation. A gap in the shape space between age classes 0–2 and 3–6 is visible. The difference between those two age clusters is most likely linked to the low sample size in age class 2 (n=2) and it is likely that the ontogenetic trajectory would form a continuum if age class 2 would contain more specimens.

The results of the mevolCVP function suggested the reduction of the dataset to the first six PCs in all subsequent analyses. Significant differences among the seven age classes (MANOVA $p < 0.001$, $F = 310.38$) allowed us to perform a CVA with a-priori defined groups (age classes 0–6) resulting in an overall classification accuracy of 85.6%. The distances in Procrustes shape space among the age stages increase as growth progresses, with a gap between the stages 0–2 and 3–6 (Supplementary Table 4).

When comparing the miniature breeds to the regular-sized ones in shape space, it becomes evident that the Shetland pony specimens align with the respective age classes of the regular breeds, but constitute the most ‘youthful’ cohorts of the respective stages (Figure 2 A). The smallest of all horse breeds, the Falabella (age class 6), clusters most closely to age stages 3 and 4 due to its rounder cranial shape. Age class 6 occupies the largest Procrustes shape space of all age classes with around 70% as determined by Foote’s partial disparity in the whole cranium (Supplementary Table 5). Age class 2 occupies the smallest shape space, due to the very small sample size (n = 2). The overall Procrustes variance of the whole cranium is 0.0030.

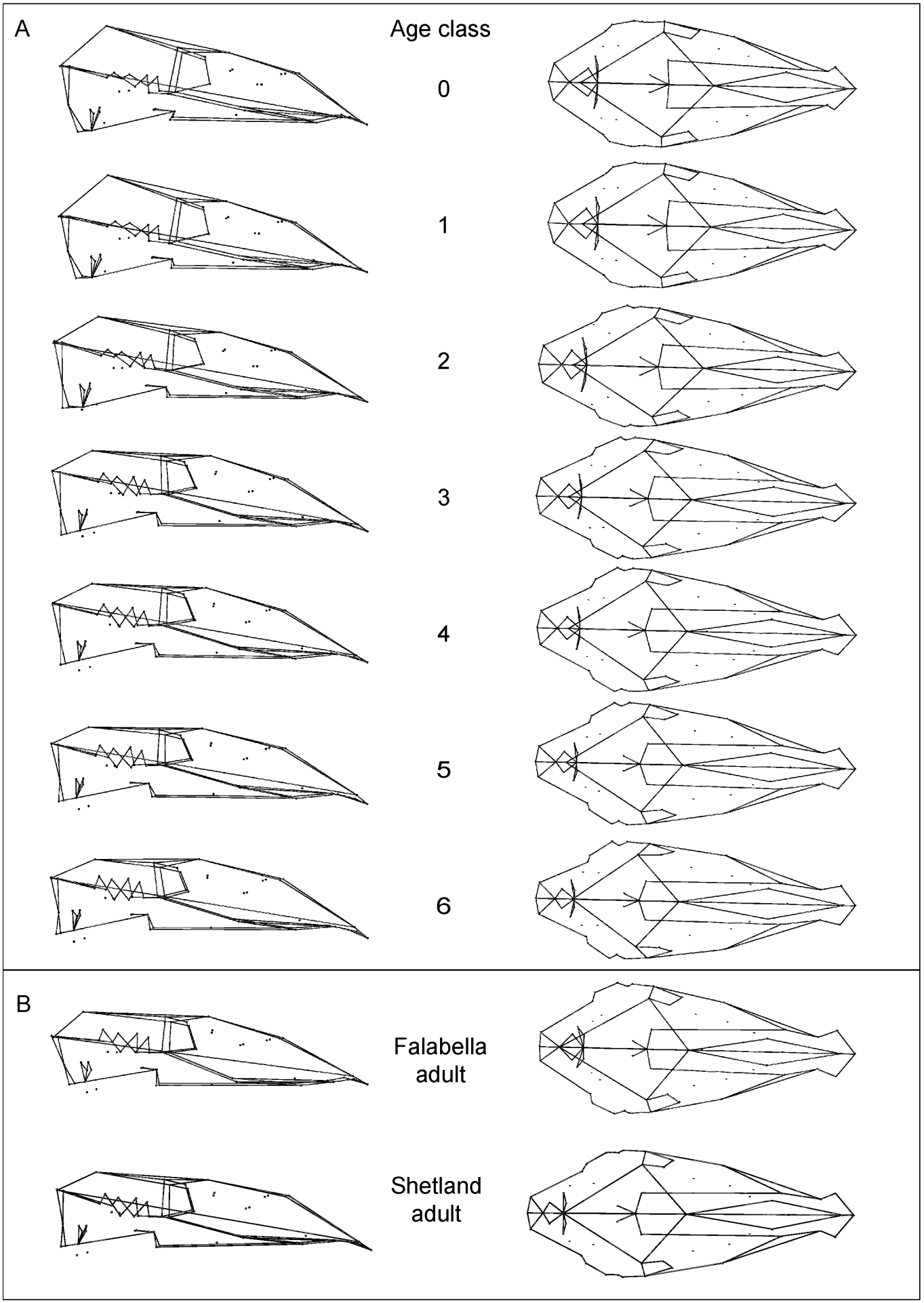
A
Age class
0
1
2
3
4
5
6
B
Falabella adult
Shetland adult

Figure 1 (left): Cranial shapes in lateral and dorsal view for A) the average shape of each age class (0 – 6) of all specimens belonging to a regular-sized breed (for detailed sample composition see Supplementary Table 1), B) the Falabella and C) the average shape of age class 6 of Shetland ponies; all crania are scaled to the same length for better comparison.

Conserved orbital module and ontogenetically variable modules

Individual PCAs based on subsets of the cranial landmarks corresponding to five cranial modules show that all analysed modules change markedly during ontogenetic development (Figure 2 B-D, F), except for the orbital (ORB) module (Figure 2 E). The cranial base (CB) and zygomatic pterygoid (ZP) module cluster specimens of the age groups 0—2 and 3—6 together. In contrast to that, the cranial vault (CV) and anterior-oral-nasal (AON) module cluster age groups 0 and 1 together, and age group 2 already clusters with more mature specimens. Also, in PCAs of landmarks belonging in a given module, the adult Falabella clusters more with the adult than with the juvenile specimens (Figure 2 B—F). The adult Falabella, nevertheless, resembles rather adolescent shapes in the ZP, AON and ORB region, whereas it takes adult shapes in CV and CB.

As already determined for the whole cranium, age class 6 occupies the largest portion of Procrustes shape space of all age classes with around 70% PD in every module (Supplementary Table 5). In the ORB module, age class 1 is the second most variable module (8.8%), whereas age class 5 (7.5 – 9.7%) is the second largest in all other modules. Age class 2 occupies the smallest shape space in each module separately, most likely due to the very small sample size (n=2). The overall Procrustes variance is highest in the AON (0.0069) and lowest in the ORB (0.0041) module when comparing each module separately (Supplementary Table 5).

Test of three common features associated with paedomorphism

During growth we observe that orbits in relation to cranial length experience negative allometry (Figure 3 A), and the basicranium becomes shorter relative to the length of the rostrum (Figure 3 B). The angle between the basicranium and palate does not differ among the different age stages (Figure 3 C). The growth pattern of Shetland ponies is similar to that of regular-sized horse breeds and do not show any signs of enlarged orbits or shortened rostra or increased braincase length in the adult stage (Figure 3 A -C). The adult Falabella exhibits relatively large orbits compared to regular-sized horses of the same age stage, but otherwise no other juvenile features regarding the rostrum or braincase are apparent (Figure 3 A-C). The predicted cranial length of the Shetland and Falabella derived from linear regression of adult cranial lengths from regular-sized breeds (Verzani 2014) is 24.6 cm and 31.1 cm respectively, which contrast to their actual lengths of 35.6 cm and about 39 cm, respectively. Both actual cranial lengths fall slightly outside of the 95% prediction interval (Figure 3 D). The cranial length at the upper prediction limit raises the question whether smaller breeds are constrained to have larger crania. Examination of the cranial length to wither height ratio in relation to breed ordered by increasing maximal wither height strengthens the impression that the adult miniature breeds have larger crania relative to wither height than their normal-sized counterparts (Figure 3 E).

Table 1: Description of morphological differences for three age classes of medium and large breeds (0,3,6) and age class 6 for both miniature breeds (Falabella, Shetland) for the studied sample by module (for a detailed sample composition see Supplementary Table 1).

	Medium and large breeds			**Miniature breeds**	
Module	**Age class 0**	**Age class 3**	**Age class 6**	**Falabella (age class 6)**	**Shetland (age class 6)**
Anterior-oral-nasal	Short, round, and narrow premaxilla; no incisors; maxilla in diastema much narrower than premaxilla; nasal and diastema are straight; diastema is short	Elongated and broader; Third pair of incisors erupted; elongated diastema; nasal bones are straight or curved, depending on breed; maxilla in diastema almost as broad as premaxilla	Elongated and broader; premaxilla-maxilla suture closed; elongated diastema; nasal is straight or curved depending on breed; maxilla in diastema almost as broad as premaxilla	Short, round, and broad premaxilla; maxilla in diastema almost as broad as premaxilla; all incisors fully erupted; nasal is concave	Elongated and broader; premaxilla-maxilla suture closed; elongated diastema; nasal is slightly convex; maxilla in diastema almost as broad as premaxilla
Orbital	Round or egg-shaped depending on individual; large compared to skull length; post-orbital margin is thin	Round or egg-shaped depending on individual; medium compared to skull length; postorbital margin has thickened	Round or egg-shaped depending on individual; small compared to skull length; postorbital margin is thick	Round or egg-shaped depending on individual; medium compared to skull length; postorbital margin is thick	Round or egg-shaped depending on individual; small compared to skull length; postorbital margin is thickened
Zygomatic-pterygoid	Frontal-zygomatic and temporal-zygomatic suture open; facial crest, zygomatic, and temporal form a straight line in lateral view	Frontal-zygomatic and temporal-zygomatic suture started to close; facial crest, zygomatic, and temporal form a straight line in lateral view	Frontal-zygomatic and temporal-zygomatic suture closed; facial crest and zygomatic form a straight line in lateral view; temporal is curved from lateral view	Frontal-zygomatic and temporal-zygomatic suture started closing; facial crest, zygomatic, and temporal form a curved line in lateral view	Frontal-zygomatic and temporal-zygomatic suture closed; facial crest and zygomatic form a straight line in lateral view; temporal is slightly curved from lateral view
Cranial base	Round; occipital condyle and paracondylar process have a similar length; basisphenoid-presphenoid and basisphenoid-occipital suture open; basilar part of the occipital is broad	Elongated and distinct; basisphenoid-presphenoid started to close and basisphenoid-occipital suture open; basilar part of the occipital is elongated; paracondylar process is slightly longer than paracondylar process	Elongated and distinct; basisphenoid-presphenoid and basisphenoid-occipital suture closed; basilar part of the occipital is elongated; paracondylar process is much longer than paracondylar process	Short and broad; basisphenoid-presphenoid and basisphenoid-occipital suture closed; basilar part of the occipital is broad; paracondylar process is longer than paracondylar process	Elongated and distinct; basisphenoid-presphenoid and basisphenoid-occipital suture closed; basilar part of the occipital is elongated; paracondylar process is much longer than paracondylar process

Table 1 (continued)

Cranial vault	Occipital is not fused to any other bone; round; frontal-parietal-occipital doming; occipital crest small	Frontal-parietal doming; occipital elongated; occipital crest more pronounced; occipital started fusing to surrounding bones	Frontal-parietal-occipital flattened; occipital elongated; occipital crest pronounced; occipital mostly fused with surrounding bones	Frontal-parietal-occipital doming; occipital elongated; occipital crest pronounced; occipital mostly fused with surrounding bones	Frontal-parietal-occipital doming; occipital elongated; occipital crest pronounced; occipital mostly fused with surrounding bones
Age classification	First post-natal stage, before the eruption of the first pair of incisors, up to 1 week old	Time after the eruption of the third pair of incisors until the eruption of the first molar, six month to one year, before sexual maturity; weaning is around 6 month of age	Last age stage after the eruption of the third molar, from 4 years on, skeletal maturity	Adult, age stage 6	Adult, age stage 6

Discussion

Horses show allometric cranial growth, as has been attested for most other domesticated species (Sánchez-Villagra *et al.* 2017), where the juvenile specimens are significantly different in cranial shape from the adult specimens. The largest shape differences in our sample can be found in the early age classes, between 1 and 2, as well as 2 and 3, the latter is most likely linked to the age of weaning at around six months. The change in diet from lactation to grazing of very nutrient poor grasses in the case of horses, requires a marked shape change of the cranium which has also been shown in other species (Hingst-Zaher *et al.* 2000; Jones and Goswami 2010; Tanner *et al.* 2010). The ORB module does not show distinct shape changes through ontogeny in regular-sized breeds. This might indicate that the orbit with which horses are born is already the functional orbit of an adult horse and relates to the importance of sight for flight animals already from birth. Flight animals are characterized by laterally positioned eyes which enable them to almost all-around vision (Linklater and Cameron 2002) and this behaviour is already essential for foals. The AON and CV change with the onset of age class 2, which is when the second pair of deciduous incisors erupts. The increase of space demand due to the second pair of incisors might correlate with the shape change of the AON. The CV already starts an anterior elongation and flattening of the anterior-dorsal part of the braincase in age class 2, which will continue until age class 6. The CB and ZP show marked shape changes coinciding with the onset of weaning at age stage 3. During that time the muscles for mastication have to be fully developed, and they co-vary with the ZP. After weaning, the normal head position is facing downwards while grazing, contrasting its previous positon

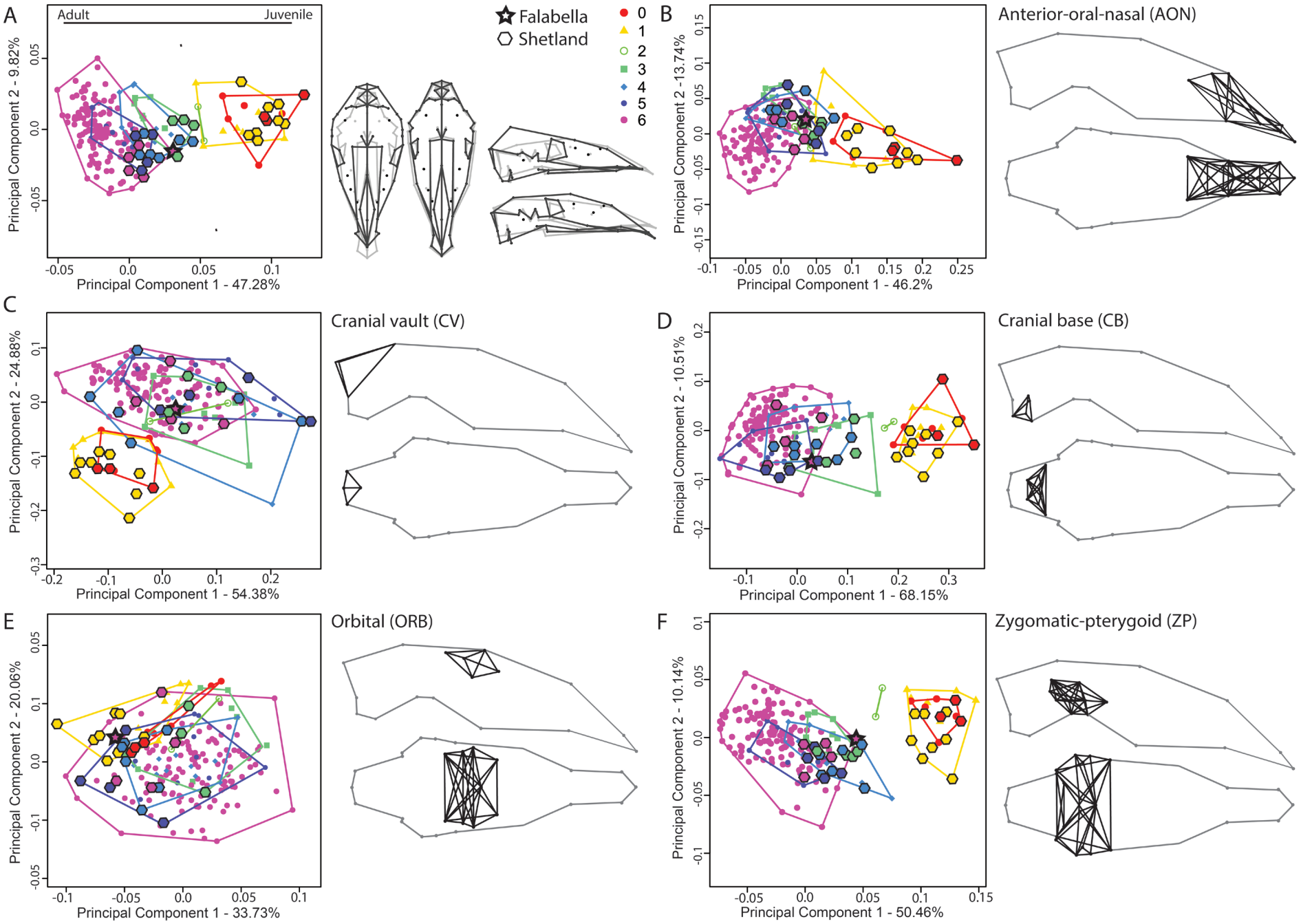
A
Adult
Juvenile
Falabella
Shetland
0
1
2
3
4
5
6
Principal Component 2 - 9.82%
Principal Component 1 - 47.28%
B
Principal Component 2 -13.74%
Principal Component 1 - 46.2%
Anterior-oral-nasal (AON)
C
Principal Component 2 - 24.88%
Principal Component 1 - 54.38%
Cranial vault (CV)
D
Principal Component 2 - 10.51%
Principal Component 1 - 68.15%
Cranial base (CB)
E
Principal Component 2 - 20.06%
Principal Component 1 - 33.73%
Orbital (ORB)
F
Principal Component 2 - 10.14%
Principal Component 1 - 50.46%
Zygomatic-pterygoid (ZP)

Figure 2 (left): Principal component analysis of 194 specimens based on A) the cranial landmark data for all modules, B) the landmark data of the anterior-oral-nasal module (AON), C) the landmark data of the cranial vault (CV), D) the landmark data of the cranial base (CB), E) the landmark data of the orbit (ORB), and F) the landmark data of the zygomatic pterygoid (ZP) with 0 — coding the dental eruption after birth, 1 — coding the eruption of the first pair of deciduous incisors, 2 — coding the eruption of the second pair of deciduous incisors, 3 —- coding the eruption of the third pair of deciduous incisors, 4 — coding the eruption of the first molar, 5 — coding the eruption of the second molar, and 6 — coding the eruption of the third molar; The two miniature breeds (Falabella – star and Shetland pony – polygon) are highlighted in shape space; in A) the lateral and dorsal view of the cranium show the shape changes along PC1 and PC2, and B) to F) the lateral and dorsal view of the cranium show the location of the respective module.

where the head was tilted upright to reach the mare's teats. The cranial base moves anteriorly with progressing growth which might hinder prolonged lactation.

The Falabella and the Shetland pony are neither miniature forms of adult regular-sized breeds, nor did they retain the juvenile features of regular-sized breeds, but do instead exhibit modular paedomorphic features, as enlarged orbits relative to cranial length, and larger crania compared to wither height, and both features are more expressed so in the Falabella than in the Shetland pony. Radinsky (1984) mentioned a strong negative allometry of orbit area and tooth row length which is reflected in the differences in proportions of miniature and regular-sized breeds by visual assessment. The initial subjective impression that the Falabella must be a paedomorphic horse probably derives from the very round anterior-dorsal part of the braincase, whose geometric morphometric description with true landmarks eluded us due to the lack of sutures in that portion of the cranium but it can be seen in the photograph of the Falabella cranium (Supplementary Figure 1). Additionally, the Falabella does exhibit a less downward curved rostrum than adults of normal-sized breeds (Figure 1). For a better assessment of a rounded cranium roof, future investigations are advised to use semi-landmarks or polygons (MacLeod 2013).

A shorter rostrum and a larger braincase are frequently stated as very distinct features for paedomorphism throughout the literature (Gould 1980; Cardini and Polly 2013; Tamagnini *et al.* 2017). We therefore tested for a shorter rostrum in adult miniature breed specimens compared to regular sized breeds. This craniofacial evolutionary allometry, where larger forms are long-faced and smaller forms show signs of paedomorphism, has recently been hypothesized to be a general pattern among mammals and is also called CREA (Cardini *et al.* 2015; Tamagnini *et al.* 2017). Previous studies on horses support this pattern by finding that the facial portion of the cranium increases exponentially, making it therefore relatively longer in larger horses (Radinsky 1984). In contrast, our data do not support the hypothesis of rostral shortening and larger braincases in miniature horse breeds. Neither the ratio of length, nor the angle between basicranium and palate, hint to paedomorphism in the two investigated miniature breeds. This might be related to strong functional constraints of the feeding apparatus, due to its high physiological and ecological significance (Radinsky 1984). Among veterinarians, it is commonly known that miniature horse breeds have a higher requirement for veterinary dentist procedures, due to their almost regular horse-sized teeth (Wilson 2012). The same health related problem has been shown for pet rabbits, which experience a rostral shortening through domestication without a change in

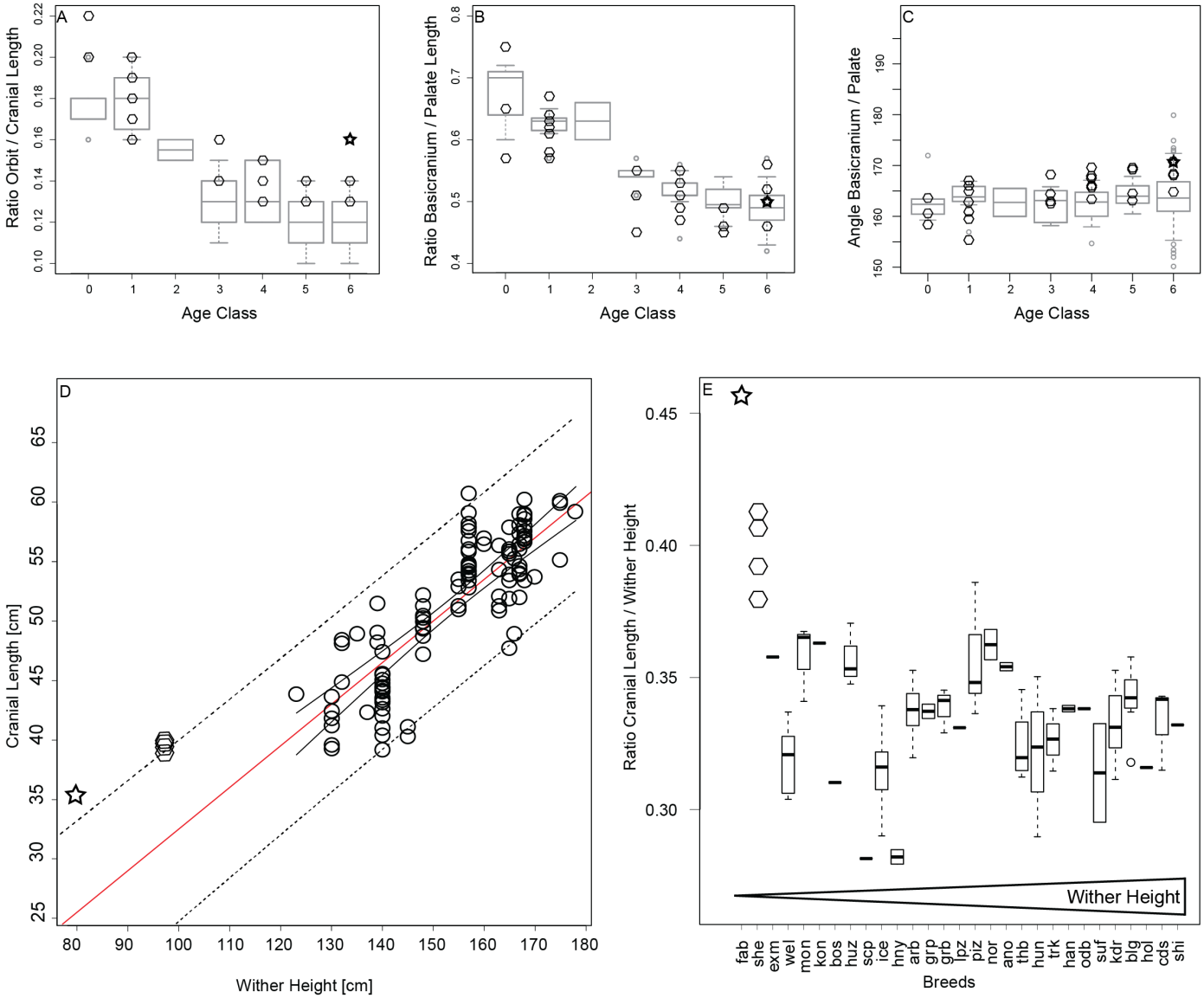

Figure 3: Test for paedomorphic cranial features in miniature horse breeds compared to different normal-sized horse breeds. (a-c) Orbit to cranial length ratio, basciranium to palate length ratio, and angle between basicranium and palate, per age category, regular-sized breeds as boxplots in grey, with Shetland ponies (diamonds) and Falabella (star) superimposed in black. (d) Adult cranial length in relation to maximal wither height with 95% confidence interval (solid line) and prediction interval (dotted line), and regression line (red). Regression line and 95% prediction curves were extended to smaller wither heights to accommodate the cranial length of the miniature breeds in the same plot. (e) Adult cranial length to wither height ratios were plotted against breed in ascending order from the smallest breed, the Falabella, to the largest breed, the Shire horse. fab: Falabella; she: Shetland pony; exm: Exmoor pony; wel: Welsh, mon: Mongolian; kon: Konik; bos: Bosnian pony; huz: Huzule; scp: Scottish pony; ice: Icelandic Horse; hny: Hackney; arb: Arab; grp: German Riding pony; grb: Grisons; lpz: Lipizzan; piz: Pinzgau; nor: Norik; ano:Anglo-Norman; thb: English Thoroughbred; hun: Hungarian; trk: Trakehner; han: Hannoverian, odb: Oldenburgian; suf: Suffolk; kdr: Kladrubian; blg: Belgian Draft; hol: Holstein; cds: Clydesdale; shi: Shire; for the ten missing breeds no information on average wither height could be found.

dentition (Böhmer and Böhmer 2017). These functional constraints have been investigated in other species before showing that miniature forms tend to have relatively larger teeth than regular sized forms (Shea and Gomez 1988). Since horses feed on a very nutrient poor diet, they are in need of a highly specialized feeding apparatus to ensure the best energy recovery possible. A strong shortening of the rostrum, as can be found in some dog and pig breeds (Geiger and Haussman 2016; Evin *et al.* 2017), is most likely possible due to their energy rich diet (carnivore/omnivore) that can be exploited with fewer, smaller, or differently placed teeth. In cows, which also feed on nutrient poor grass, one case of rostral shortening is known: the Niata breed (Veitschegger 2017): this was likely possible due to the more efficient uptake of nutrients through rumination. To our knowledge, marked rostral shortening is not known for any herbivore, non-ruminant mammal species.

The last paedomorphic feature tested in our study is the ratio of cranial length to body size. It is generally accepted that juvenile individuals of many species have a relatively large head compared to their body and limbs (Gould 1980). In horses, however, foals show very long limbs and a small but broad head (Habermehl 1975; Goodwin *et al.* 2007; Goodwin *et al.* 2008). The long legs in horses are a necessity for surviving, since the new born foals are very precocial and need to keep up with the rest of the herd from day one. Our study did not compare the actual limb to head length ratio which has been proposed as a sign for paedomorphism (Goodwin *et al.* 2007; Goodwin *et al.* 2008), but used wither height as a proxy for size. The expected cranial size for the two miniature breeds, which was calculated based on our sample set of regular sized breeds, is lower than the actual cranial size. Our data clearly show that both miniature breeds have a larger head compared to their breeds' average wither height than expected. The relatively enlarged head is a common feature of paedomorphism in many species (Gould 1980) but does not resemble the juvenile state of horses.

The shape changes involved in the selection for extreme small size in horses resulted from a combination of neomorphism and paedomorphism, the same patterns reported also for wolf (Geiger *et al.* 2017) and boar (Evin *et al.* 2017) domestication. A preliminary investigation of the ontogenetic trajectories of Shetland Ponies and Welsh hints towards prenatally developed shape differences between the two breeds (Supplementary Figure 3, not presented due to small sample size).

In conclusion, the visual assessment of not only the miniature breeds' cranial shapes, but their complete (cranial and post-cranial) morphological appearance that left us with an impression of paedomorphism could not be completely confirmed by our quantitative results. The subjective impression of paedomorphism might be based rather on features of the postcranial architecture, namely general small size or short limbs, or behavioural aspects such as the use of body language (Goodwin *et al.* 1997) or facial expressions (Waller *et al.* 2013).

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Chapter IV - Supplement

Supplementary Table 1: The raw data table is provided in electronic form at the end of this thesis due to its large size.

Supplementary Table 2: Description of the landmarks, including position, type, and assignment to a module, collected on each cranium; Type I: discrete juxtapositions of tissue types and Type II: maxima of curvature or other local morphogenetic processes (Rohlf and Bookstein 1990); Modules are anterior oral-nasal (AON), cranial base (CB), cranial vault (CV), orbital (ORB), zygomatic pterygoid (ZP), and not applicable to a module (NA), Goswami, 2006).

Cranium	Position	Type	Module
1 - 2	Posterior tip of the upper third incisor	II	AON
3 - 4	Posterior most point of the nasal-premaxilla suture	I	AON
5 - 6	Premaxillary-maxillary-nasal suture	I	AON
7 - 8	Dorsoposterior tip of the infraorbital foramen	II	AON
9 - 10	Anterior tip of the facial crest	II	NA
11	Nasion, nasal-frontal suture, midline	I	ORB
12 -13	Junction of the lacrimal, maxilla, and nasal sutures	I	ORB
14 - 15	Zygo-lacrimal suture on the orbital margin	I	ORB
16 - 17	Lacrimal-frontal suture on the orbital margin	I	ORB
18 -19	Supraorbital foramen	II	ORB
20 - 21	Anterior tip of the zygo-temporal suture	I	ZP
22 - 23	Posterior tip of the zygo-temporal suture	I	ZP
24 - 25	Dorsal tip of the frontal-temporal suture	I	ZP
26 - 27	Ventroposterior tip of the zygomatic process	II	ZP
28 - 29	Dorsalmost point of the vertically orientated posterior margin of the zygomatic process	II	ZP
30 - 31	Ventrolateralmost point of squamous part of temporal bone	II	ZP
32	Anterior tip of the occipital triangle	I	CV
33	Posterior tip of the nuchal crest	II	CV
34 - 35	Dorsolateral tip of the nuchal crest	II	CV
36	Dorsalmost point on the margin of the foramen magnum	II	CV
37	Point between first incisors from ventral side	II	AON
38 - 39	Posteriormost tip of the premaxillary-maxillary suture, ventral	I	AON
40 - 41	Anterior tip of the second premolar	II	AON
42	Posteriormost point of the incisive canal	II	AON
43	Posterior tip of the palatine process of the incisive bone	I	AON
44	Posterior tip of the palatine-palatine suture	I	NA
45 - 46	Distal tip of the pterygoid hamulus	II	NA
47 - 48	Anterior tip of the caudal alar foramen	II	ZP
49	Posterior tip of the vomer on the midline	II	ZP
50 - 51	Medial tip of the mandibular fossa	II	ZP
52 - 53	Canal for hypoglossal nerve	II	CB
54 - 55	Fossa medial of the paracondylar process	II	CB
56 - 57	Distal tip of the paracondylar process	II	CB
58	Ventral tip of the foramen magnum	II	CB
59 - 60	Posteriormost tip of the occipital condyle	II	CB

Supplementary Table 3: Average wither height in cm for each breed with reference.

Breed	Average (cm)	Reference
Anglo Norman	160	Stud-Book Selle Français (2017)
Arab	148	Arabian Horse Association (2017)
Belgian Draft	168	Trekpaard (2017)
Bosnian Pony	137	Gesellschaft der Freunde (2017)
Clydesdale	175	Clydesdale Horse Society (2017)
Exmoor Pony	123	The Exmoor Pony Society (2017)
Falabella	78	The Falabella Miniature Horse Association (2017)
Grison	155	Pferdezucht-Genossenschaft Graubünden (2017)
German Riding Pony	148	Deutsche Reiterliche Vereinigung (FN) (2017)
Hannoverian	165	Hannoveraner Verband (2017)
Hackney	145	American Hackney Horse Society (2017)
Holstein	170	Verband der Züchter des Holsteiner Pferdes e.V. (2017)
Hungarian	165	Bene *et al.* (2014)
Huzule	139	Polish Horse Breeders Association (2017a)
Icelandic Horse	140	International Federation of Icelandic Horse Associations (2017)
Kladrubian	167	Stud Kladruby nad Labem (2017)
Konik	135	Polish Horse Breeders Association (2017b)
Lipizzan	155	Zuchtorganisation Gestüt Lipica (2017)
Mongolian	132	Wikipedia (2017)
Norik/ Pinzgauer	157	Druml *et al.* (2008)
Nonius	160	Nonius Lótenyésztő Országos Egyesület (2017)
Oldenburgian	165	Oldenburger Verband (2017)
Scottish pony	140	IG Highland Pony "Der Clan" e.V. (2017)
Shetland Pony	97	Schweizerischer Shetlandponyverband (2017)
Shire	178	Shire Horse Society (2017)
Suffolk	166	Suffolk Horse Society (2017)
English Thoroughbred	163	The Jockey Club (2017)
Trakehner	165	Trakehner Verband (2017)
Welsh	130	The Welsh and Cob Society (2017)

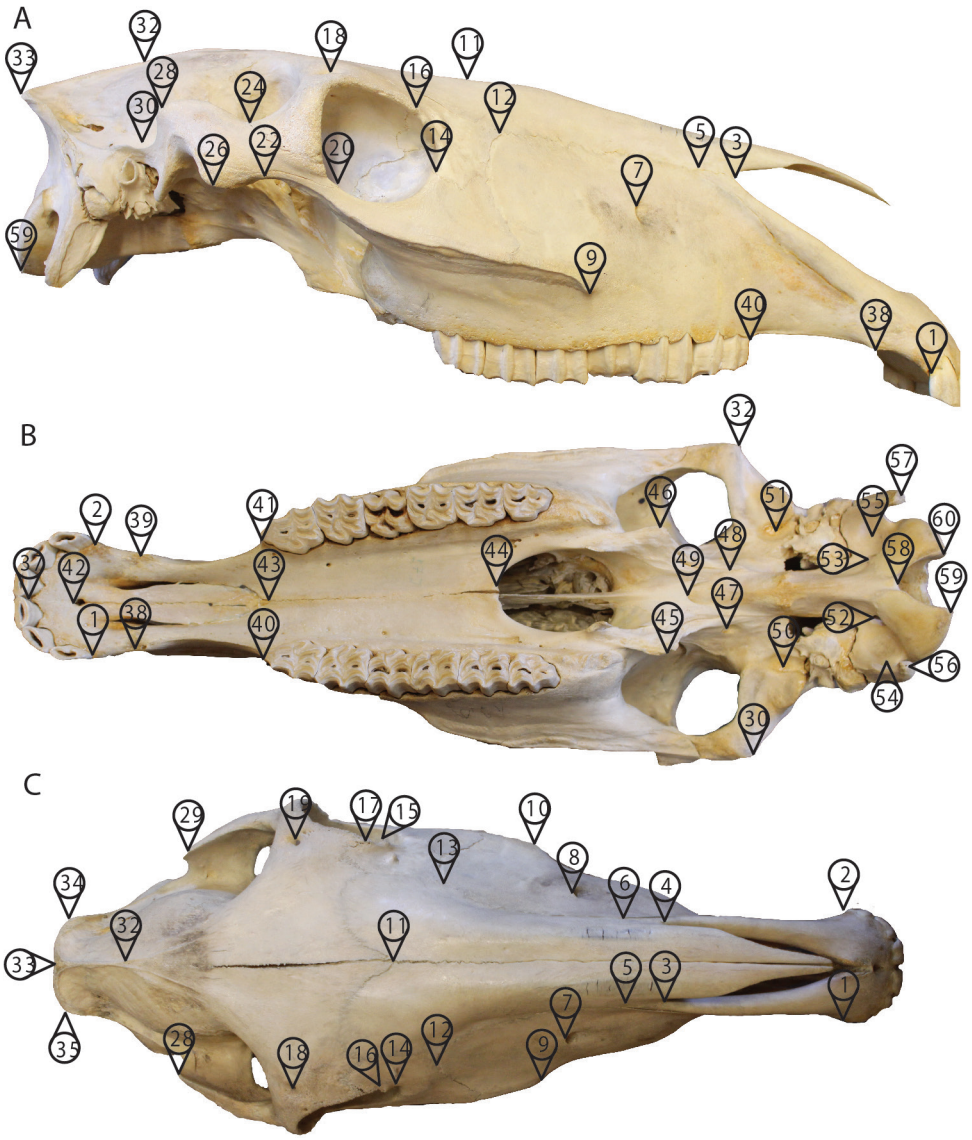
Supplementary Table 4: Mahalanobis distance for the cranium according to dental age class: 0 - coding the dental eruption after birth, 1 - coding the eruption of the first pair of deciduous incisors, 2 - coding the eruption of the second pair of deciduous incisors, 3 - coding the eruption of the third pair of deciduous incisors, 4 - coding the eruption of the first molar, 5 - coding the eruption of the second molar, and 6 - coding the eruption of the third molar.

	0	**1**	**2**	**3**	**4**	**5**
1	0.76					
2	4.02	3.56				
3	8.51	7.89	5.37			
4	10.30	9.66	7.42	2.31		
5	12.34	11.69	9.61	4.76	2.60	
6	12.38	11.71	9.65	5.26	3.50	1.90

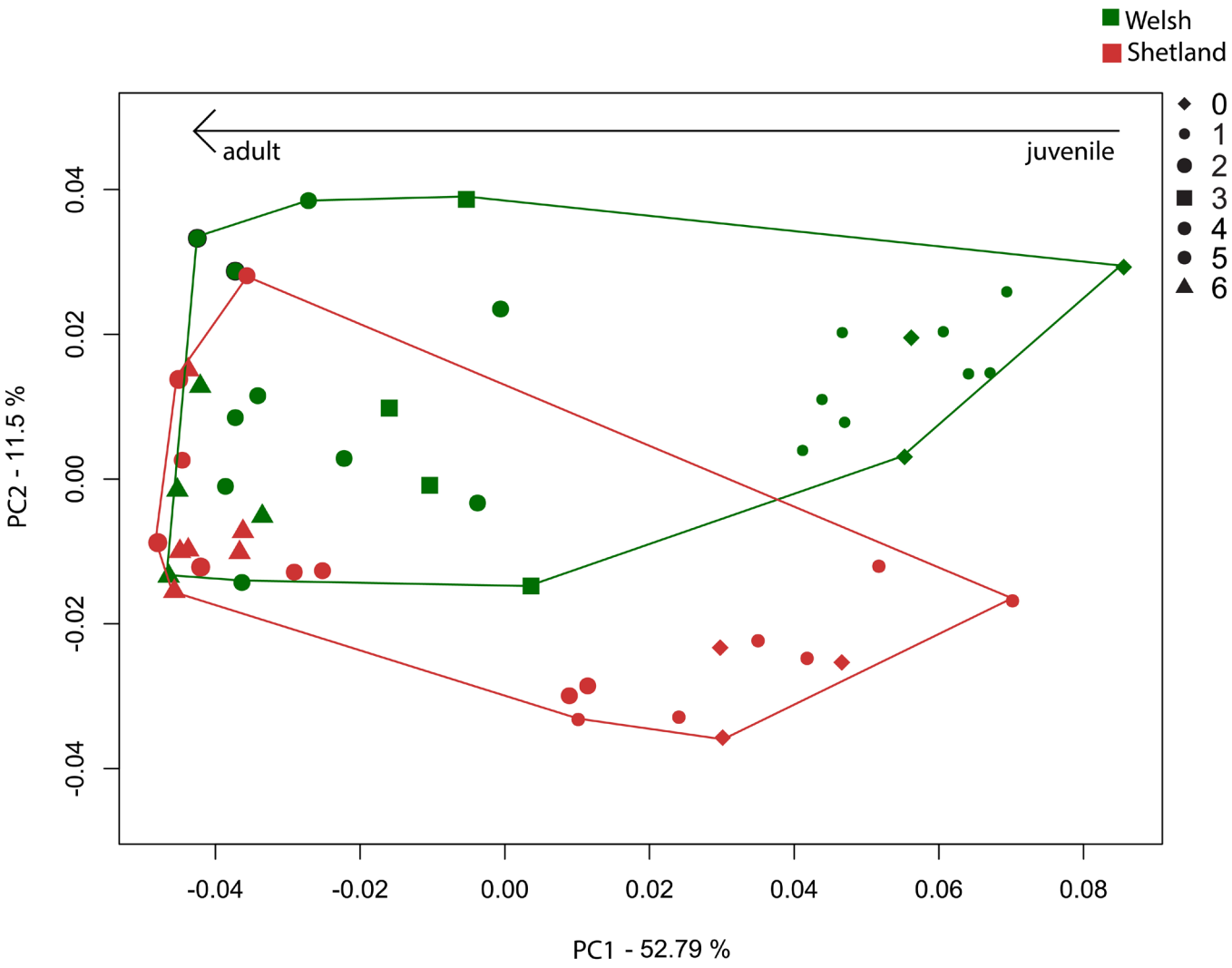
Supplementary Table 5: Partial disparity for all modules and each module separately for all seven age classes (0-6); Modules are anterior oral-nasal (AON), cranial base (CB), cranial vault (CV), orbital (ORB), zygomatic pterygoid (ZP), and not applicable to a module (NA); 0 - dental eruption after birth, 1 - eruption of the first pair of deciduous incisors, 2 - eruption of the second pair of deciduous incisors, 3 - eruption of the third pair of deciduous incisors, 4 - eruption of the first molar, 5 - eruption of the second molar, and 6 - eruption of the third molar.

	0 (n=8)	**1 (n=15)**	**2 (n=2)**	**3 (n=10)**	**4 (n=16)**	**5 (n=14)**	**6 (n=129)**	**Overall Procrustes variance**
All Modules	2.6	5.1	1.0	3.7	6.3	9.2	72.1	0.0030
AON	3.1	5.9	0.8	3.4	7.5	8.8	70.6	0.0069
CB	3.1	5.7	0.8	3.4	7.5	9.1	70.3	0.0060
CV	3.5	5.0	2.1	3.0	6.8	7.5	72.0	0.0065
ORB	3.2	8.8	1.6	3.9	8.5	7.9	66.1	0.0041
ZP	2.1	6.4	1.0	4.4	6.9	8.4	70.9	0.0049

Supplementary Figure 1: Examples of different cranial shapes during ontogeny from lateral view if available for each age class (0-6) for Falabella and Shetland ponies (miniature breeds) and Welsh (regular sized breed); each stage is represented by a different individual and all crania are scaled to the same length for comparison.



Supplementary Figure 2: A total of 60 landmarks used in this study shown on the A) lateral B) ventral C) dorsal side of the cranium of a normal sized horse breed (for a detailed description of the landmarks see Supplementary Table LM).



Supplementary Figure 3: Principal component analysis of ontogenetic series of Shetland pony (red) and Welsh (green) specimens.

Conclusion and Perspectives

Studies on differences in developmental rate or timing can provide valuable insights on the generation of morphological disparity among and within domesticated species. This thesis investigated differences among horse breeds and between domesticated horses and wild equids and which developmental patterns might be related to them.

Chapter 1 showed that breed affiliation has an influence on the variation of gestation length in horses, whereas body size does not correlate with gestation length. The data could not provide an answer to why the average gestation length is longer in some breeds than in others. Studies on prenatal development in horses are difficult to undertake, however, a closer investigation on embryonic development, using ultrasound or scanning techniques, might shed light on this topic. A review on size differences in neonates showed that Shetland ponies, a miniature horse breed, are born with around 13.3% of their adult weight while in a medium breed, like the Arab it is 9.8%, and in the largest breed, the Shire, 6.8% (Platt 1984). The analyses in Chapter 1 did not result in significant differences in average gestation length between Shetland ponies and Arabs, hinting towards differences in prenatal growth rate to generate these weight differences. In a side project, with the help of a bachelor student, I investigated possible differences in postnatal growth rate (Box 1) among different breeds. During the literature research for Chapter 1, I came across the statement that the variation in gestation length in horses is larger than in other domesticated species (Bos and Van der Mey 1980; Aoki *et al.* 2013). As the results of Chapter 1 show, gestation length is influenced by domestication. Hence, stricter controlled production animals, such as cattle, pigs, sheep, and goats, should have an economically favorable lower variation in gestation length than non-production animals (horses, dogs, rabbits, cats). Results of this second chapter support this hypothesis. The stricter breeding control in pigs, cattle, and sheep, significantly decrease the variation in their gestation length. Goats are an exception to this rule, which is likely due to their less constrained breeding standards (Asdell 1929). Domestication might have increased or decreased the variability of gestation length in some species, while a secondary restriction occurs through strict controlled breeding in the farming industry.

Chapters 3 and 4 of my thesis are concerned with the morphological variation related to domestication. In Chapter 3, I assessed, quantified, and evaluated the disparity of cranium, mandible, and teeth of domesticated horses in comparison with all extant wild equids. The cranial, mandibular, and dental shape variation of domesticated horses supports Darwin's hypothesis (1868), by exceeding the shape variation of the wild equids. The cranial shapes of domesticated horses group with the Przewalski's horses and tend to separate from zebras and donkeys, suggesting a relationship of cranial shape and phylogenetic relatedness. The lower values of integration in domesticated horses than in wild equids were associated with higher disparity values across the six confirmed cranial modules, hinting to disparity enabled by modularization and relaxed co-variation among them. Chapter 4 focused on the intraspecific disparity among different sized horse breeds in an ontogenetic context. I showed that the standing hypothesis of novel shape generation in miniature horse breeds through paedomorphism is only partially true and that modular paedomorphic features such as enlarged orbits relative to cranial length and larger crania compared to wither height generate shape differences. With the

Box 1: Comparative bone histology among adult horses

The body size differences among horse breeds are remarkable (ranging from 70 cm to 2 m in wither height). However, the growth patterns generating such variation are not known. The study of bone histology and bone growth marks (BGMs) yields valuable insights into the life history of an organism (Marín-Moratalla *et al.* 2013; Kolb *et al.* 2015). BGMs record cyclic variation in bone tissue deposition as zones, lines of arrested growth (LAGs) and annuli, the latter two indicating an interruption or decrease in cortical bone apposition (Sander and Andrássy 2006). The number of cyclical growth marks (CMGs) within a bone cortex can provide insight into longevity, age at maturity, or growth rate (among others Chinsamy and Valenzuela (2008); (Köhler 2010)). As the femur is one of the best suited bones for skeletochronology in equids (Nacarino-Meneses *et al.* 2016b), we aim to assess differences in growth rates among femora of small and large breeds, by comparing the distances between LAGs (Marín-Moratalla *et al.* 2013; Kolb *et al.* 2015). First, we identify and count the LAGs and measure the thickness of the growth zones, and second, we identify differences in bone tissue composition among the small and regular-sized breeds.

A total of seven left femora from different sized horse breeds with known age at death (Supplementary Table 1) were sampled. All samples were provided by the local veterinary clinic (Vetsuisse Faculty, University of Zurich). Following the standard procedures of our lab (Kolb et al. 2015), we prepared histological sections from the mid-shaft of each bone. We extracted a 3 cm thick slice from each femur and degreased it in boiling water and soap before embedding it with epoxy resin (Araldite). After cutting and grinding (Chinsamy and Raath 1992), we studied the thin sections using a Leica DM 2500 M composite microscope equipped with Leica DFC 420 C digital camera and the Leica IM 50 Image Manager®.

We did not find distinct variation among different sized horse breeds in bone histology or BGMs (Figure 1). The primary tissue type of all sampled specimens is plexiform fibro-lamellar bone. All sections further show a high amount of remodeling with secondary osteons replacing the primary bone, primarily in the anterior cortex. In some parts the primary bone was completely replaced by Haversian tissue. High mechanical stress during speed gates explains a higher amount of osteons in the anterior part of the section (Firth 2006; Zedda *et al.* 2008; Nacarino-Meneses *et al.* 2016a).

The high amount of remodeling, however, complicated the identification of LAGs (shown as an example in Figure 1 D). In all specimens, we counted fewer LAGs than expected from known age. Hence, we did not proceed with further analyses on these samples. The low visibility or absence of LAGs is probably based on the strong remodeling of the bone and the resorption of the inner bone cortex by the medullar cavity (Sander and Andrássy 2006; Nacarino-Meneses *et al.* 2016b), the latter process erasing much of the growth record of the individual equid. Additionally, the domestic lifestyle and year round food supply might influence the formation of LAGs, since in wild equids BGMs are easier to identify and agree with the estimated age of the specimens (Nacarino-Meneses *et al.* 2016b). Since horses experience rapid growth in the first two years of their lives (Frape 1986; Stover *et al.* 1992), ontogenetic series of different sized horse breeds might provide further insight on changes of bone tissue composition and might facilitate the identification of BGMs.

Box 1 (continued)

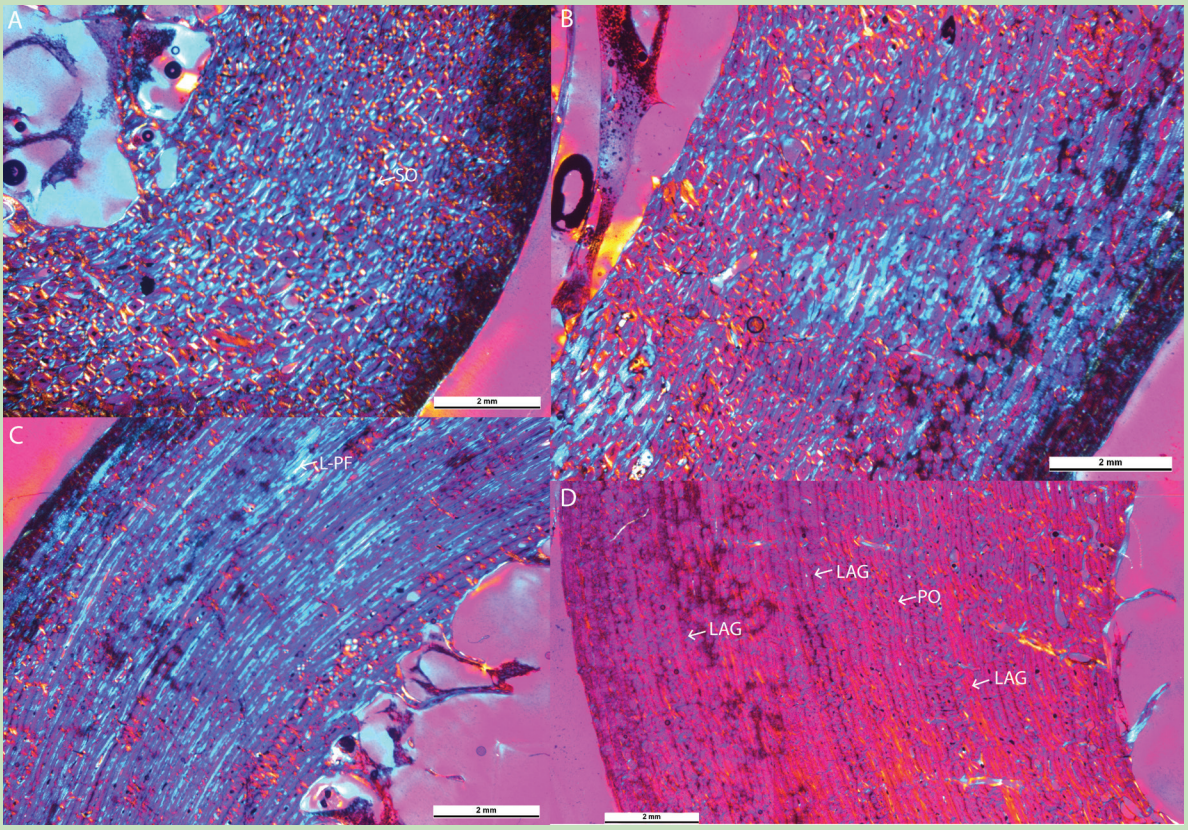


Figure 1: Detail of the thin sections from the anterior (A and B) and posterior (C and D) of a small (A and C; Icelandic horse) and a large horse breed (B and D; Dutch Warmblood). Note that LAGs in the cortical bone are mostly obscured by bone remodeling processes. PO: primary osteon, SO: secondary osteon, LAG: line of arrested growth (assumed), L-PF: lamellar/parallel-fibered bone; all images were obtained under polarized light with a ¼ λ filter.

help of two Bachelor students, I started a preliminary investigation of the relationship of differences in suture closure and cranial shape in different breeds (Box 2).

The results of my thesis clearly show that domestication strongly influences the life history and morphology of horses. Details on how differences in developmental timing and rate are connected to the generation of disparity needs further analyses with comprehensive life history data for at least a few morphologically distinct breeds, e.g. a miniature, a medium, and a large breed. All chapters of my thesis emphasize the importance of long term data acquisition by breeders and access to carefully curated collections. Especially in species with long gestation length and high longevity, gathering sufficient amounts of data for profound analyses is difficult without long-term engagements and collaborations. Notwithstanding the high abundance in horses today, collection material is scarce and has mostly been gathered in the 19th century. Additional information for each specimen, such as wither height, age, or breed affiliation, was rarely available. Furthermore, the lack of ontogenetic series, not only for domesticated horses but also for the Przewalski's horse as a comparison, complicated the investigation of the questions raised during my PhD thesis. Further studies on the changes in developmental timing and rate related to domestication will largely benefit from additional data recorded by breed on the timing of different life history

Box 2: Cranial suture closure pattern in domesticated horses

Differences in timing of suture closure during development can generate differences in cranial shape of domesticated mammals (Geiger and Haussman 2016). Sutures allow bone growth, facilitate articulation and deformation, and absorb mechanical stress (Cohen 1993). During two block course projects (2015 with Fabienne Barmettler and 2017 with Angela Hellingman), we scored and compared closure states of 16 different sutures (Figure 1 A and B), to investigate differences in relative closure values of sutures among different horse breeds and wild equids. Closure states were calculated by scoring all individuals and dividing the sum of all scores by the number of individuals. During the first project in 2015, Fabienne Barmettler showed that in a direct comparison of a zebra specimen and a domesticated horse specimen, both ten years of age, more sutures are closed in the zebra specimen. To provide a more significant result, further analyses with ontogenetic series of both groups are necessary. In the second project in 2017, Angelica Hellingman recapitulated the study including different breeds and one zebra species. The results of this more comprehensive study contradict the previous project and show no differences in suture closure states between the domesticated horse breeds and the zebra.

This box will further only present a subset of breeds (and no wild equids, Supplementary Table 1), since the full dataset still needs to be analyzed. Comparing closure states of fully grown individuals of six different sized breeds (Figure 2 A) showed a similar closure pattern in all breeds. Sutures located in the anterior portion of the skull, e.g., praemaxilla-maxilla or lacrimal-square, show a higher variation

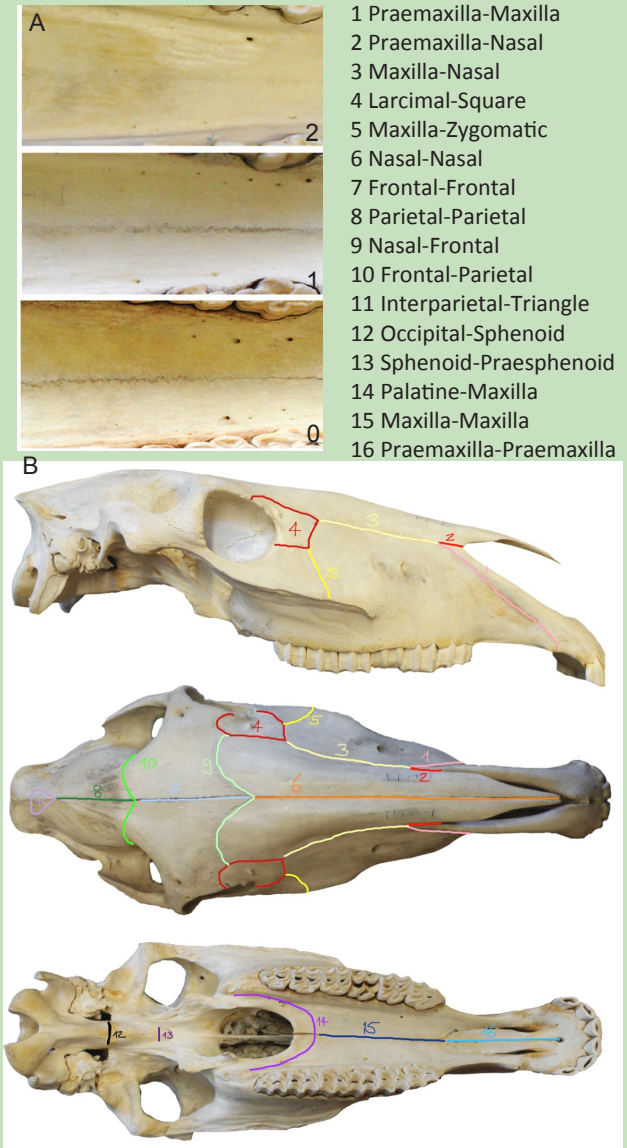
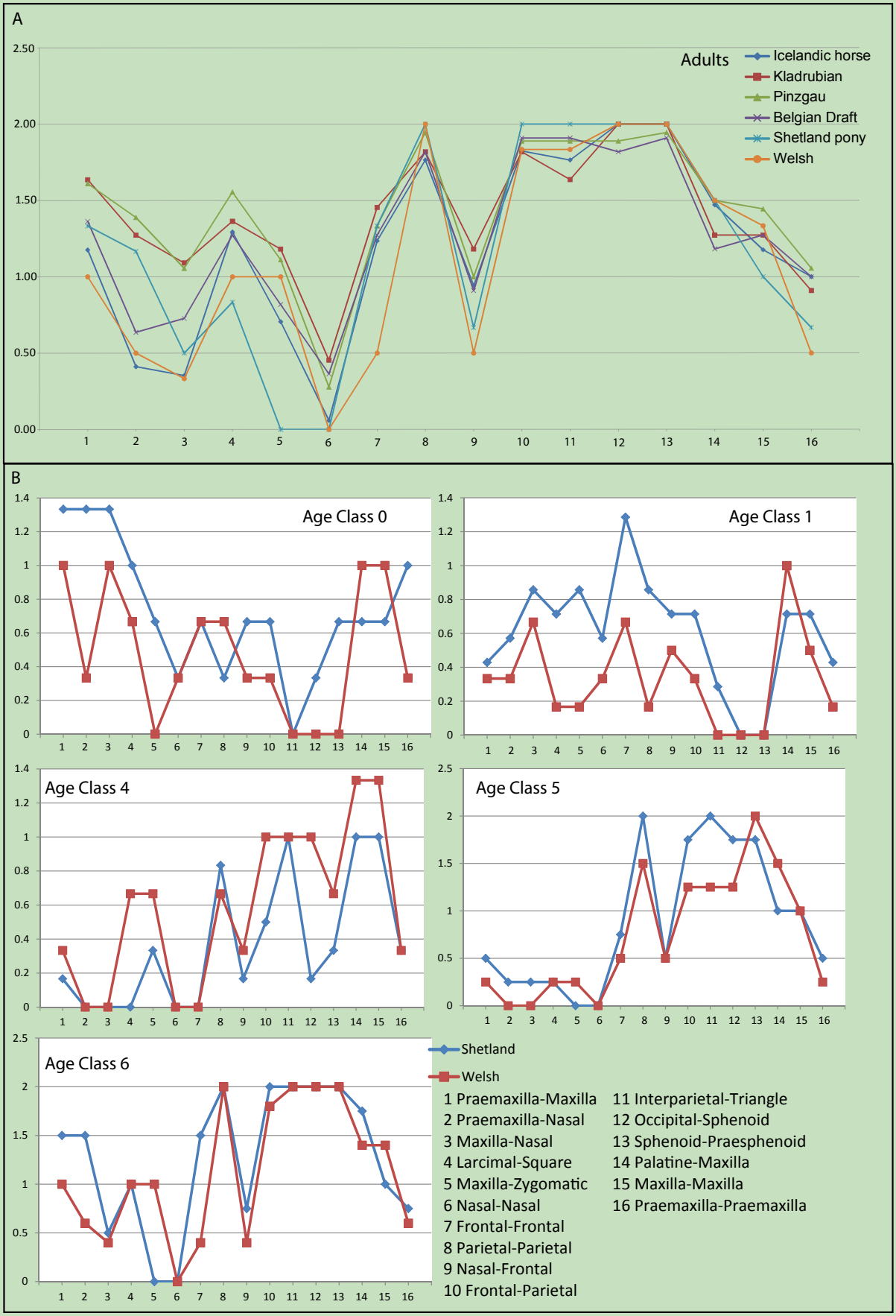


Figure 1: A) Suture closure states with 0-open, 1-closing, 2-closed, and B) 16 different sutures scored in the study presented on the cranium from lateral, dorsal, and ventral view.

in closure scores than those located in the posterior portion of the skull. One explanation might be that the higher level of mechanical stress from chewing requires higher flexibility. A more detailed comparison of the Shetland pony, a miniature breed with a peculiar skull shape, to a regular sized breed, the Welsh, showed differences in closure scores among juvenile age stages but a similar pattern in adult individuals (Figure 2 B). The earlier closure of some sutures in the miniature breed, such as the frontal-parietal or frontal-frontal in age class 1, might be linked to their

Box 2 (continued)



Box 2 (continued)

paedomorphic appearing domed shape of the anterior-dorsal part of the basicranium (see Chapter 4 for details). For a more detailed analysis a larger dataset has to be collected for ontogenetic series of different sized breeds.

Figure 2 (left): Closure scores for 16 different sutures in A) crania of adult specimens of six horse breeds: Icelandic horse (n=17), Kladrubian (n=12), Pinzgau (n=18), Belgian Draft (n=11), Shetland pony (n=4), and Welsh (n=5), and B) crania of Shetland ponies (blue, n=19) and Welsh (red, n=16) individually per age class (0 - dental eruption after birth, 1 - eruption of the first pair of deciduous incisors, 4 - eruption of the first molar, 5 - eruption of the second molar, and 6 - eruption of the third molar).

variables (age of weaning, age of sexual maturity, age of skeletal maturity, longevity) in combination with data to assess growth rates (size at birth, size at sexual maturity, size at skeletal maturity). Good alternatives to collections could be provided by close collaborations with breeders and veterinary clinics using methods such as CT-scanning, ultrasounds, or x-rays.

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Conclusion and Perspectives - Supplement

Supplementary Table 1: Additional information on the sample composition of the seven studied specimens; F: female, G: gelding.

Breed	Gender	Age (years)	Cause of Death	ID	Length (cm)
Pony (undefined)	F	25	Euthanized	P_F_010190	30.5
Dutch Warmblood	F	28	Euthanized	HW_F_020487	52
Icelandic horse	G	25	Euthanized	IS_MK_010190	36.5
English Thoroughbred	G	6	Euthanized	EV_MK_010109	49
Hanoverian	G	16	Euthanized	HAN_MK_010199	44
Inländer	F	17	Euthanized	IN_F_140498	47
Polish Warmblood	F	7	Euthanized	PW_F_010108	50

Supplementary Table 2: Raw data including individuals and scored sutures (1 Praemaxilla-Maxilla, 2 Praemaxilla-Nasal, 3 Maxilla-Nasal, 4 Larcimal-Square, 5 Maxilla-Zygomatic,6 Nasal-Nasal,7 Frontal-Frontal, 8 Parietal-Parietal, 9 Nasal-Frontal,10 Frontal-Parietal, 11 Interparietal-Triangle, 12 Occipital-Sphenoid, 13 Sphenoid-Praesphenoid, 14 Palatine-Maxilla, 15 Maxilla-Maxilla, 16 Praemaxilla-Praemaxilla, see Figure 1 in Box 2) with A) including only adult specimens and B) including specimens of different age stages.

Breed	ID	Suture															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Icelandic	B_0001328_H_ice_P_3_6	1	0	0	1	0	0	1	2	1	2	2	2	2	1	1	1
Icelandic	B_0001339_H_ice_P_3_6	1	0	0	1	0	0	1	1	1	2	2	2	2	1	2	1
Icelandic	B_0001340_H_ice_P_3_6	1	0	0	1	0	0	1	2	1	2	2	2	2	2	1	1
Icelandic	B_0001381_H_ice_P_3_6	1	0	0	1	1	0	1	2	1	2	2	2	2	2	1	1
Icelandic	B_0001382_H_ice_P_3_6	1	0	0	1	0	0	1	2	1	2	2	2	2	2	1	1
Icelandic	B_0001383_H_ice_P_3_6	2	1	1	2	2	0	2	2	1	2	2	2	2	2	2	1
Icelandic	B_0001385_H_ice_P_3_6	1	0	0	1	0	0	0	1	0	1	1	2	2	1	1	1
Icelandic	B_0001387_H_ice_P_3_6	1	0	0	1	0	0	1	2	1	2	2	2	2	1	1	0
Icelandic	B_0001388_H_ice_P_3_6	1	0	0	1	0	0	1	2	0	2	1	2	2	1	1	1
Icelandic	B_0001390_H_ice_P_3_6	1	0	1	2	1	0	2	2	1	2	2	2	2	2	1	1
Icelandic	B_0001392_H_ice_P_3_6	1	0	0	2	1	0	2	2	1	2	2	2	2	1	0	1
Icelandic	B_0001393_H_ice_P_3_6	1	0	0	1	0	0	1	1	1	1	1	2	2	1	1	1
Icelandic	B_0001394_H_ice_P_3_6	2	2	1	2	2	0	2	2	1	2	2	2	2	2	2	1
Icelandic	B_0001395_H_ice_P_3_6	1	0	0	0	0	0	0	1	0	1	1	2	2	0	0	0
Icelandic	K_0001760_H_ice_P_3_6	1	0	1	2	2	0	2	2	2	2	2	2	2	2	2	2
Icelandic	K_0001851_H_ice_P_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2
Icelandic	K_0002217_H_ice_P_3_6	1	2	0	1	1	0	1	2	1	2	2	2	2	2	1	1
Kladrubian	V_00E1027_H_kdr_W_4_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	1
Kladrubian	V_00E321_H_kdr_W_3_6	0	0	0	0	0	0	1	1	0	0	0	2	2	0	0	0
Kladrubian	V_00E322_H_kdr_W_3_6	1	0	0	0	0	0	1	2	0	2	1	2	2	2	1	1
Kladrubian	V_00E337_H_kdr_W_3_6	2	2	2	2	2	1	1	2	2	2	2	2	2	2	2	1
Kladrubian	V_00E340_H_kdr_W_3_6	2	1	0	1	1	0	1	2	0	2	2	2	2	0	0	1
Kladrubian	V_00E341_H_kdr_W_3_6	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	1
Kladrubian	V_00E342_H_kdr_W_3_6	2	1	0	2	0	0	1	2	1	2	2	2	2	1	1	1
Kladrubian	V_00E357_H_kdr_W_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	1	1	1
Kladrubian	V_00E358_H_kdr_W_3_6	1	0	0	0	0	0	1	1	0	2	1	2	2	0	1	1
Kladrubian	V_00E661_H_kdr_W_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	1
Kladrubian	V_0000E73_H_kdr_W_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	1
Pinzgau	V_00E1295_H_piz_C_3_6	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	1
Pinzgau	V_00E1298_H_piz_C_3_6	1	2	0	1	0	0	1	2	0	2	2	2	2	2	1	1
Pinzgau	V_00E1299_H_piz_C_3_6	1	1	0	1	0	0	1	2	0	2	2	2	2	1	2	1
Pinzgau	V_00E362_H_piz_C_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	1
Pinzgau	V_00E363_H_piz_C_3_6	2	2	2	2	2	0	1	2	2	2	2	2	2	2	2	1
Pinzgau	V_00E364_H_piz_C_3_6	2	2	1	2	2	0	2	2	1	2	2	2	2	1	1	1
Pinzgau	V_00E366_H_piz_C_3_6	2	0	1	2	2	1	2	2	2	2	2	2	2	2	1	1
Pinzgau	V_00E367_H_piz_C_3_6	1	0	0	1	0	1	1	2	0	1	1	2	2	1	1	1

Supplementary Table 2 (continued)

Breed	ID	Suture															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Pinzgau	V_0000E68_H_piz_C_3_6	2	2	2	2	1	0	1	2	1	2	2	2	2	1	1	1
Pinzgau	V_0000E69_H_piz_C_3_6	2	2	2	2	1	0	1	2	1	2	2	2	2	1	1	1
Pinzgau	V_0000E71_H_piz_C_3_6	2	1	0	1	0	0	1	2	0	2	2	2	2	1	1	1
Pinzgau	V_000E930_H_piz_C_3_6	1	0	0	1	1	0	1	2	1	2	2	2	2	1	1	1
Pinzgau	V_000E931_H_piz_C_3_6	1	0	0	1	0	0	1	2	0	2	2	2	2	1	1	1
Pinzgau	V_000E943_H_piz_C_3_6	0	1	0	0	0	0	1	1	0	2	1	1	1	1	1	0
Pinzgau	V_000E944_H_piz_C_3_6	2	2	1	2	1	1	1	2	1	2	2	2	2	2	2	1
Pinzgau	V_000E945_H_piz_C_3_6	2	2	2	2	2	0	2	2	2	1	2	1	2	2	2	1
Pinzgau	V_000E946_H_piz_C_3_6	2	2	2	2	2	0	1	2	1	2	2	2	2	2	2	2
Pinzgau	V_000E950_H_piz_C_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2
Belgian	H_00Eblg1_H_blg_C_3_6	1	0	0	0	0	0	1	2	0	2	2	1	2	1	1	1
Belgian	H_00Eblg2_H_blg_C_3_6	2	1	1	2	1	1	2	2	1	2	2	2	2	1	1	1
Belgian	H_00Eblg3_H_blg_C_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2
Belgian	H_00Eblg5_H_blg_C_3_6	1	0	0	1	0	0	1	1	0	2	2	2	2	1	1	0
Belgian	H_00Eblg7_H_blg_C_3_6	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	1
Belgian	H_000E966_H_blg_C_3_6	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0
Belgian	H_000E967_H_blg_C_3_6	1	0	0	1	0	0	1	2	1	2	2	2	2	1	1	1
Belgian	H_000E968_H_blg_C_3_6	1	0	0	1	0	0	1	2	1	2	2	2	2	1	1	1
Belgian	H_000E969_H_blg_C_3_6	1	0	0	1	1	0	1	2	0	2	2	2	2	1	1	1
Belgian	H_000E970_H_blg_C_3_6	2	1	2	2	1	1	2	2	2	2	2	2	2	2	2	2
Belgian	H_000E971_H_blg_C_3_6	2	1	1	2	2	0	1	2	1	2	2	2	2	1	2	1
Shetland	K_0016437_H_she_P_3_6	1	1	1	1	0	0	2	2	0	2	2	2	2	2	1	0
Shetland	K_0016438_H_she_P_3_6	1	1	0	1	0	0	1	2	1	2	2	2	2	1	1	1
Shetland	K_0023613_H_she_P_3_6	2	2	1	1	0	0	1	2	1	2	2	2	2	2	1	1
Shetland	V_00E1025_H_she_P_3_6	2	2	0	1	0	0	2	2	1	2	2	2	2	2	1	1
Welsh	K_0016719_H_wel_P_3_6	2	2	1	2	2	0	1	2	1	2	2	2	2	2	2	1
Welsh	K_0018146_H_wel_P_3_6	1	0	1	1	1	0	1	2	1	2	2	2	2	1	2	1
Welsh	K_0019782_H_wel_P_3_6	1	1	0	1	1	0	0	2	0	2	2	2	2	2	1	0
Welsh	K_0019937_H_wel_P_3_6	1	0	0	1	1	0	0	2	0	2	2	2	2	1	1	1
Welsh	K_0024678_H_wel_P_3_6	0	0	0	0	0	0	0	2	0	1	2	2	2	1	1	0

B

Breed	ID	Age Class	Suture													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Shetland	K_0016428_H_she_P_0_0	0	2	2	2	1	1	1	2	1	2	1	0	1	2	2	1	2
Shetland	K_0020465_H_she_P_0_0	0	1	2	2	1	1	0	0	0	0	0	0	0	0	0	1	1
Shetland	V_00E1061_H_she_P_0_0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Shetland	K_0016187_H_she_P_1_1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	0

Supplementary Table 2 (continued)

Breed	ID	Suture															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Shetland	K_0016509_H_she_P_1_1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0
Shetland	K_0017500_H_she_P_1_1	1	0	0	0	0	1	0	1	2	1	2	1	0	0	1	1
Shetland	K_0017502_H_she_P_1_1	1	0	0	1	1	0	0	1	1	1	0	0	0	0	1	1
Shetland	K_0017654_H_she_P_1_1	1	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0
Shetland	K_0018712_H_she_P_1_1	1	1	2	2	1	0	1	2	2	1	1	0	0	0	1	1
Shetland	K_0022033_H_she_P_1_1	1	0	1	2	1	2	2	2	1	1	1	1	0	0	0	0
Shetland	K_0017164_H_she_P_2_4	4	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0
Shetland	K_0016326_H_she_P_2_4	4	0	0	0	0	1	0	0	2	0	1	1	0	1	1	0
Shetland	K_0017799_H_she_P_2_4	4	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0
Shetland	K_0017800_H_she_P_2_4	4	0	0	0	0	0	0	0	1	0	1	2	0	1	1	1
Shetland	B_0001298_H_She_P_2_4	4	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0
Shetland	V_000E345_H_she_P_2_4	4	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1
Shetland	K_0016449_H_she_P_2_5	5	1	0	0	0	0	0	1	2	1	2	2	2	2	0	1
Shetland	K_0017268_H_she_P_2_5	5	0	0	0	0	0	0	1	2	1	2	2	2	1	1	1
Shetland	K_0017290_H_she_P_2_5	5	1	1	1	1	0	0	1	2	0	2	2	2	2	2	1
Shetland	K_0017906_H_she_P_2_5	5	0	0	0	0	0	0	0	2	0	1	2	1	2	1	0
Shetland	V_00E1025_H_she_P_3_6	6	2	2	0	1	0	0	2	2	1	2	2	2	2	2	1
Shetland	K_0023613_H_she_P_3_6	6	2	2	1	1	0	0	1	2	1	2	2	2	2	2	1
Shetland	K_0016437_H_she_P_3_6	6	1	1	1	1	0	0	2	2	0	2	2	2	2	2	0
Shetland	K_0016438_H_she_P_3_6	6	1	1	0	1	0	0	1	2	1	2	2	2	2	1	1
Welsh	K_0016574_H_wel_P_0_0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0
Welsh	K_0016700_H_wel_P_0_0	0	1	1	1	1	0	1	1	1	0	1	0	0	0	1	1
Welsh	K_0018405_H_wel_P_0_0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	1	0
Welsh	K_0016188_H_wel_P_1_1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Welsh	K_0016575_H_wel_P_1_1	1	1	0	1	0	0	1	1	0	1	0	0	0	0	1	1
Welsh	K_0016576_H_wel_P_1_1	1	0	1	1	0	0	0	1	0	1	0	0	0	0	1	0
Welsh	K_0016720_H_wel_P_1_1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0
Welsh	K_0017827_H_wel_P_1_1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
Welsh	K_0018711_H_wel_P_1_1	1	0	0	1	0	0	0	1	0	0	1	0	0	0	2	1
Welsh	K_0017170_H_wel_P_2_4	4	1	0	0	1	0	0	0	1	1	1	1	1	0	1	1
Welsh	K_0017291_H_wel_P_2_4	4	0	0	0	0	1	0	0	0	0	1	1	1	2	1	1
Welsh	K_0019157_H_wel_P_2_4	4	0	0	0	1	1	0	0	1	0	1	1	1	0	2	2
Welsh	K_0016426_H_wel_P_2_5	5	0	0	0	0	0	0	0	0	1	1	1	0	2	1	1
Welsh	K_0016718_H_wel_P_2_5	5	1	0	0	1	1	0	1	2	1	2	1	2	2	2	1
Welsh	K_0017173_H_wel_P_2_5	5	0	0	0	0	0	0	0	2	0	0	1	1	2	1	1
Welsh	K_0017919_H_wel_P_2_5	5	0	0	0	0	0	0	1	2	0	2	2	2	2	2	1
Welsh	K_0016719_H_wel_P_3_6	6	2	2	1	2	2	0	1	2	1	2	2	2	2	2	2

Supplementary Table 2 (continued)

Breed	ID	Suture															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Welsh	K_0018146_H_wel_P_3_6	6	1	0	1	1	1	0	1	2	1	2	2	2	2	1	2	1
Welsh	K_0019782_H_wel_P_3_6	6	1	1	0	1	1	0	0	2	0	2	2	2	2	2	1	0
Welsh	K_0019937_H_wel_P_3_6	6	1	0	0	1	1	0	0	2	0	2	2	2	2	1	1	1
Welsh	K_0024678_H_wel_P_3_6	6	0	0	0	0	0	0	0	2	0	1	2	2	2	1	1	0

Appendix

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Research

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On the lack of a universal pattern associated with mammalian domestication: differences in skull growth trajectories across phylogeny

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As shown in a taxonomically broad study, domestication modifies postnatal growth. Skull shape across 1128 individuals was characterized by 14 linear measurements, comparing 13 pairs of wild versus domesticated forms. Among wild forms, the boar, the rabbit and the wolf have the highest proportion of allometric growth, explaining in part the great morphological diversity of the domesticated forms of these species. Wild forms exhibit more isometric growth than their domesticated counterparts. Multivariate comparisons show that dogs and llamas exhibit the greatest amount of differences in trajectories with their wild counterparts. The least amount is recorded in the pig–boar, and camel and horse pairs. Bivariate analyses reveal that most domesticated forms have growth trajectories different from their respective wild counterparts with regard to the slopes. In pigs and camels slopes are shared and intercepts are different. There is a trajectory extension in most domesticated herbivores and the contrary pattern in carnivorous forms. However, there is no single, universal and global pattern of paedomorphosis or any other kind of heterochrony behind the morphological diversification that accompanies domestication.

1. Introduction

Genomic and archeological studies have helped to establish with great certainty the wild species from which domesticated mammals originated [1], and many of the genetic bases of some of the traits that arise with domestication and selective breeding have been discovered [2–4]. Phenomic studies are lagging behind, although these would be fundamental to understand what at the end fascinates us today as it fascinated Darwin as well: the morphological diversity (disparity) in domesticated forms. Here an ontogenetic perspective is fundamental, given its centrality to understand the evolution of form [5].

The power of selective breeding to produce morphological diversity, as best exemplified by the case of dogs, is uncontested [6–9]. But are there intrinsic aspects in the biology of the dog that make it a particularly plastic species? How do dogs compare with other domesticated forms? To answer these questions, we need to compare the fundamental pattern of differentiation, of development, that characterizes the species to be compared.

In the case of mammals, much of the differentiation among species occurs during the postnatal period, although many species-specific features are already established at birth [10,11]. The most widely used marker of morphological diversity is the skull, given its complexity in form (shape and size) and embryological origin (mesodermal and neural crest; pharyngeal arches, dermatocranium and endocranium; [12]), and its relation to organs such as the brain, to sensory organs and to feeding function. Studies of skulls benefit from the fact the museum collections keep specimens that are available for study, in some cases of populations no longer existing in the wild.

We present a comprehensive examination of skull growth trajectories in 13 wild versus domesticated forms of mammals, including members of all major clades in which domestication has occurred (figure 1). By using a similar method and measurements protocol, we can for the first time investigate the similarities and differences in the changes produced by domestication.

Although a synthetic and comparable quantification of morphospace occupation among domesticated mammals is still in its infancy, previous works [8,15] have stated what newer methods are likely to confirm: some species have become more morphologically diverse than others [9]. Dogs and pigs, for example, are more morphologically diverse than cats and horses [16]. What is behind these patterns? Our study addresses the potential effects of growth patterns in the diversification associated with domestication, with a dataset that leaves no ambiguity about comparisons across species and that is broad in taxonomic scope.

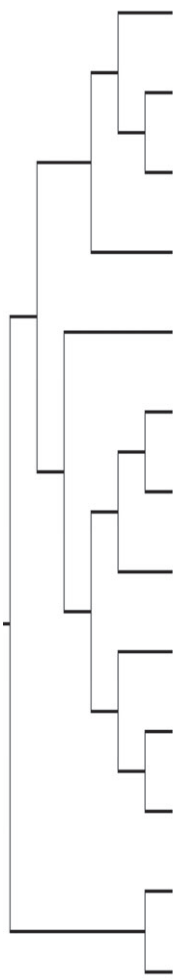
2. Material and methods

Our study of extensive growth series (figure 1) is based on 1128 specimens deposited in 15 institutions in eight countries (electronic supplementary material, 1). In each species we aimed at having individuals evenly distributed in size and representing as much as possible of the postnatal growth trajectory. For all species we considered young specimens with deciduous teeth or incomplete adult dentition, with the smallest specimens being 50% the size or less than the larger specimens. We took 14 skull measurements that serve to optimize the characterization of the skull shape for a broad sample of species (figure 2; electronic supplementary material, 1).

In many of the studied species, domestication has been estimated to have started between 10 000 and 5000 years ago, dogs having been domesticated earlier and rabbits and minks in much more recent times [1]. It would be ideal to have access to growth series of the original wild forms, at the time of the beginning of their domestication, but this is obviously impossible. We then sampled the best approximation to it: modern wild individuals when possible from the geographic area of origin of domestication (electronic supplementary material, 1). We sampled domesticated individuals from populations that do not represent specialized breeds (e.g. short snouted varieties), but instead a more generalized domesticated form, thus the best approximation to the fundamental aspect of domestication and not the result of intense selective breeding for some specific trait.

For ontogenetic pattern comparisons, we performed both multivariate and bivariate analyses. The former considers size as a latent variable affecting all measured variables simultaneously and is thus more realistic, whereas bivariate approach is suitable for statistical comparison of slopes and intercepts of regressions for wild versus domestic forms.

In our bivariate and multivariate approach, we pooled all ages together assuming a uniform growth rate, until reaching the final adult size (i.e. offset of ontogenetic regression). The mode of growth of



Domestic common name (*n*)	**Domestic scientific name**	**Wild common name** (*n*)	**Wild scientific name**
Dog (101)	*Canis lupus familiaris*	Grey wolf (24/23)	*Canis lupus*
Ferret (59)	*Mustela putorius furo*	European polecat (57)	*Mustela putorius putorius*
American mink (14)	*Neovison vison*	American mink (61)	*Neovison vison letifera*
Cat (134)	*Felis silvestris catus*	Wildcat (40)	*Felis silvestris lybica*
Horse (63/69)	*Equus ferus caballus*	Przewalski's horse (17)	*Equus ferus przewalskii*
Goat (49)	*Capra hircus*	Bezoar (15)	*Capra aegagrus*
Sheep (43)	*Ovis aries*	Mouflon (31)	*Ovis musimon*
Pig (42)	*Sus scrofa domestica*	Wild boar (44/45)	*Sus scrofa scrofa*
Bactrian camel (10/8)	*Camelus bactrianus*	Bactrian camel (11)	*Camelus ferus*
Llama (20)	*Lama glama*	Guanaco (27/28)	*Lama guanicoe*
Alpaca (14)	*Lama pacos*	Vicuña (29)	*Vicugna vicugna*
Rabbit (24)	*Oryctolagus cuniculus* f. domesticus	European rabbit (90)	*Oryctolagus cuniculus*
Guinea pig (43)	*Cavia porcellus*	Brazilian guinea pig (47)	*Cavia aperea*

Figure 1. Pairs of domesticated versus wild forms investigated in this work. The number of specimens investigated is indicated in brackets, for both multivariate and bivariate analyses; in case they differ they are listed first and second, respectively. Names of taxa followed most common current use (e.g. [13]).The names of the species provided fits current use, although some of it is not universal and is indeed controversial [14].

some carnivorans (e.g. pinnipeds), which exhibit specialized social behaviour, includes a second spurt in adult males, as reflected in significant differences in young and adult males trajectories linked to extreme sexual dimorphism (e.g. [17]). Such condition was not observed in previous analyses in terrestrial carnivorans [18,19], even considering large felids [19] and polygamous herbivores [20]. Such evidence assumes non-significant differences in young and adult growth trajectories. The same can be assumed with respect to sexual dimorphism, in which several reports suggest the same growth trajectories for both sexes in terrestrial carnivores and herbivores (e.g. [18,20]).

2.1. Multivariate approach

The multivariate approach used (e.g. [21]) evaluated the first (unit-scaled) eigenvector of a principal component analysis (PCA) based on a variance–covariance matrix of log10 transformed data for all

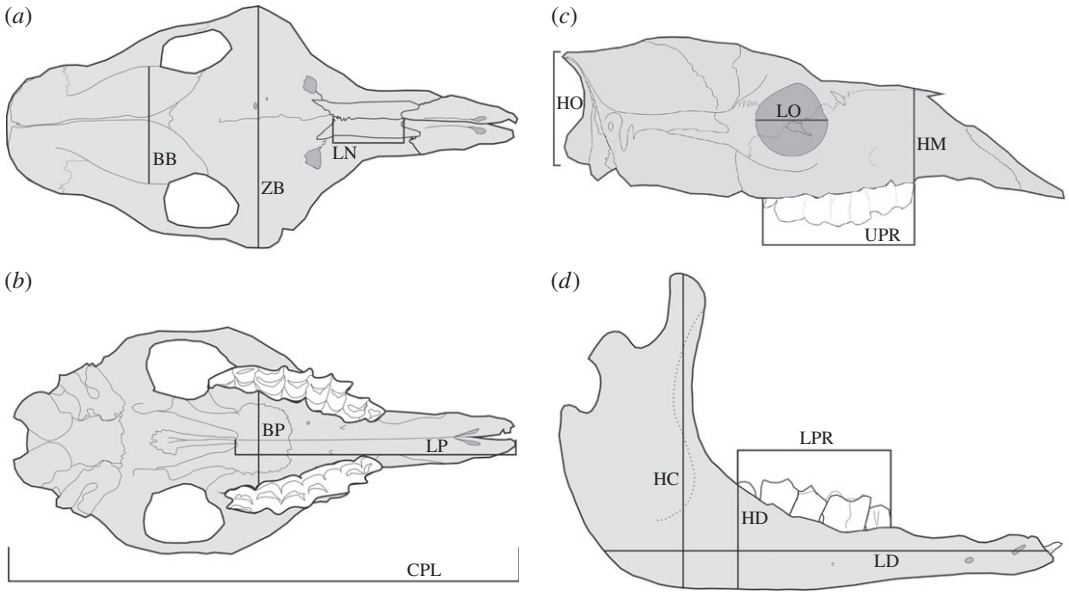


Figure 2. Measurements of the skull in dorsal (*a*), ventral (*b*) and lateral (*c*) views and the mandible (*d*). BB, breadth of the braincase; BP, breadth of palate; CPL, condylo-premaxillary length; HC, height of the coronoid process; HD, height of the dentary; HM, height of the muzzle; HO, height of occipital plate; LD, length of the dentary; LN, length of the nasals; LO, length of the orbit; LP, length of palate; LPR, length of lower post-canine row; UPR, length of upper post-canine or of molariform tooth row; ZB, zygomatic breadth. See electronic supplementary material, 1, for details.

variables and for each taxon [22]. For a given variable, allometry is the statistical deviation of its corresponding eigenvector element from the hypothetical isometric value (i.e. if the global growth pattern is size invariant), which is calculated as $1/p^{0.5}$ with p equal to the number of variables. In order to generate confidence intervals for each of the empirically derived first-eigenvector elements, statistical deviation from isometry was estimated using the application of jackknife [23]. The generated confidence interval may be inclusive of the isometric value and, therefore, indistinguishable from isometry, or it may exclude such value and, therefore, be considered significantly allometric (i.e. positive or negative, with a higher or lower rate of change for the specific variable when compared with overall growth). From the collection of n pseudovalues obtained from a resampling strategy, in which one specimen by PCA round is eliminated in the sample [23], mean and standard deviation were calculated for each element corresponding to one skull variable. The mean represents the raw jackknife estimate of the multivariate allometry coefficient for that variable. The difference between this estimate and the actual value from the complete sample is a measure of bias [24]. Confidence intervals may be severely influenced by extreme pseudovalues (those obtained by the resampling just described) and trimming the m largest and the m smallest values decreased the standard deviations and allowed for better allometric estimations [24]. We report untrimmed as well as ($m = 1$) trimmed calculations of confidence intervals, opting for the results with either lower average standard deviation or bias. The statistical analyses (PCA + jackknife resampling) were programmed in R [25]; the script is available on request.

The multivariate coefficients of allometry are expressed as confidence intervals. They can show different signs in their allometric trend (positive and negative allometry) and no intersection in their extreme values, showing an absolute value in their difference defined as the distance between the higher and lower limits of both intervals. Similarly, the two compared confidence intervals derived from multivariate analyses can show the same allometric trend, but with no overlap in values. In other cases, both compared intervals can show intersection in their values, and thus no absolute differences, but one of them including the hypothetical value of isometry ($1/p^{0.5}$, 0.267 for this study), showing in consequence different sign in their allometric trends. In other cases, both intervals can share the same allometric sign with intersection of their values.

In order to quantify the changes between wild and domesticated forms (confidence intervals), we added the individual change of all variables as an 'added change' for each comparison between forms, and also the 'added change' for each cranial variable across our 13 comparisons (table 1).

Table 1. Comparisons of growth trajectories between domesticated and wild forms, based on multivariate analysis of 14 linear measurements of the skull and dentary bone. In each cell we report the difference between the confidence intervals of the rate of growth (i.e. allometric coefficients) obtained for each variable and for each species/form (see Material and methods). The cells in dark grey show the greatest amount of change, those with the value in 0 in light grey indicate the cases in which the difference in the growth trend exists, although the confidence intervals (CI) overlap (intersect). We treated the growth trajectory (allometric) values as continuous characters and we subtracted the amount of change between each wild–domestic pair. For CI choice based on trimmed or untrimmed sample of pseudovalues see Material and methods and electronic supplementary material, 3. The question marks result from the lack of data on the dentaries of goats. We added the individual values for all variables as 'added change' for each species/form, and also for the same variable for all species. Abbreviations as in figure 2.

forms	minor bias	CPL	LN	HM	UPR	LP	BP	LO	ZB	BB	HO	LD	HD	HC	LPR	added change
Canis lupus familiaris	untrimmed	0.009	0	0.056	0.062	0.002	0	0	0.1	0.02	0.04	0	0.013	0.002	0.103	0.41
Canis lupus lupus	trimmed															
Mustela putorius furo	trimmed	0.017	0.083	0	0.054	0	0	0	0	0	0	0	0	0	0	0.15
Mustela putorius putorius	untrimmed															
Neovison vison	untrimmed	0	0	0	0	0	0.036	0	0	0	0	0	0	0	0	0.04
Neovison vison letifera	untrimmed															
Felis silvestris catus	untrimmed	0.007	0	0	0	0	0	0.013	0.001	0	0	0	0.042	0.034	0	0.10
Felis silvestris lybica	trimmed															
Equus ferus caballus	untrimmed	0.002	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Equus ferus przewalskii	untrimmed															
Capra hircus	untrimmed	0	0	0	0	0	0	0	0	0	0	?	?	?	?	0.00
Capra aegagrus	untrimmed															
Ovis aries	untrimmed	0	0	0	0	0.003	0	0	0	0	0	0	0	0	0	0.00
Ovis musimon	untrimmed															
Sus scrofa domestica	untrimmed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Sus scrofa scrofa	untrimmed															
Lama glama	untrimmed	0.028	0.061	0	0	0.007	0.035	0.033	0.011	0	0.032	0.033	0.037	0	0	0.28
Lama guanicoe	trimmed															
Lama pacos	trimmed	0.011	0.011	0	0.033	0	0	0	0	0	0	0.019	0	0.082	0	0.16
Vicugna vicugna	untrimmed															
Camelus bactrianus	untrimmed	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Camelus ferus	trimmed															
Oryctolagus cuniculus f. domesticus	trimmed	0.001	0	0.013	0	0.034	0.016	0	0	0	0	0	0.001	0.0120		0.08
Oryctolagus cuniculus	untrimmed															
Cavia porcellus	untrimmed	0	0.006	0	0	0	0	0	0	0.015	0	0	0	0	0.036	0.06
Cavia aperea	untrimmed															
added change		0.08	0.16	0.07	0.15	0.05	0.09	0.05	0.11	0.04	0.07	0.05	0.09	0.13	0.14	

2.2. Bivariate approach

We compared proportions of different cranial parts across species. Ontogeny is expressed as a linear regression, in which the time frame is implicitly incorporated (size proxy), in order to describe relative modifications as the individuals grow and examine potential heterochronic processes. We interpreted coefficients of allometry as growth rates (e.g. [26,27]). Overall size was estimated as the geometric mean [28], which ensures the isometric condition of the independent variable. The relation of each variable to overall size was examined using the standard allometric equation derived from a power growth function converted to its (base10) logarithm [29].

We performed *F*-tests with the null coefficient set at 1.0 to assess deviations from isometry [27,30], after corroborating that the independent variable (i.e. geometric mean) was normally distributed (Shapiro–Wilk test, electronic supplementary material, 1). We examined differences in ontogenetic regressions comparing slopes and intercepts of the linear trajectories (table 2). Significance level was set to $p = 0.0036$; that *p*-value representing the usual 5% alpha level divided by the number of statistical tests (14 regressions) performed over the same sample units (Bonferroni correction; [31]). For those regressions that exhibit the same slopes and intercepts, we evaluated the existence of 'shifts', meaning any significant extensions or truncations of the domestic trajectory with respect to the wild one. For such comparisons we applied standardized major axis regression (SMA; [27]) and followed Warton *et al*. [32] in order to test the common SMA slope using a χ^2 distribution [30]. In those cases where domesticated and wild forms shared a common slope, we compared the significance of the common *y*-intercepts using the Wald test (as described in [32]). All regression coefficients, statistical parameters, and tests were performed using the smatr package in R [30]. In our comparison of growth trajectories between domesticated and wild forms, we considered the wild form as the ancestor of the domesticated one, from which a heterochronic pattern may derive or not in the domesticated form. Following Reilly *et al*. [33], peramorphosis (extended development) is produced by an increase in rate (acceleration, larger slope in the domesticated than in the wild form), a later offset time (hypermorphosis, trajectory extension in the domesticated form), or an earlier onset time (pre-displacement of the trajectory in the domesticated form). Conversely, paedomorphosis (results in traits produced by truncated development) is produced by a slower rate (deceleration, small slope), an earlier offset time (hypomorphosis, trajectory truncation in the domesticated form) or a later onset time (post-displacement of the trajectory in the domesticated form). Changes in the intercept would either indicate neomorphy or—together with changes in slope and/or onset and offset time—be indicative of heterochronic changes.

3. Results

Based on the multivariate comparison of growth trajectories between wild and domesticated forms, the following patterns emerge (table 1). Wolves–dogs and llamas–guanacos are the pairs that exhibit the greatest amount of added change (0.407 and 0.277, respectively). In fact, the dog is more diverging from wolf than are cats and horses from their wild counterparts (they exhibit values of 0.097 and 0.002, respectively). The species with the least amount of differences wild versus domesticated are the pig, camel and goat.

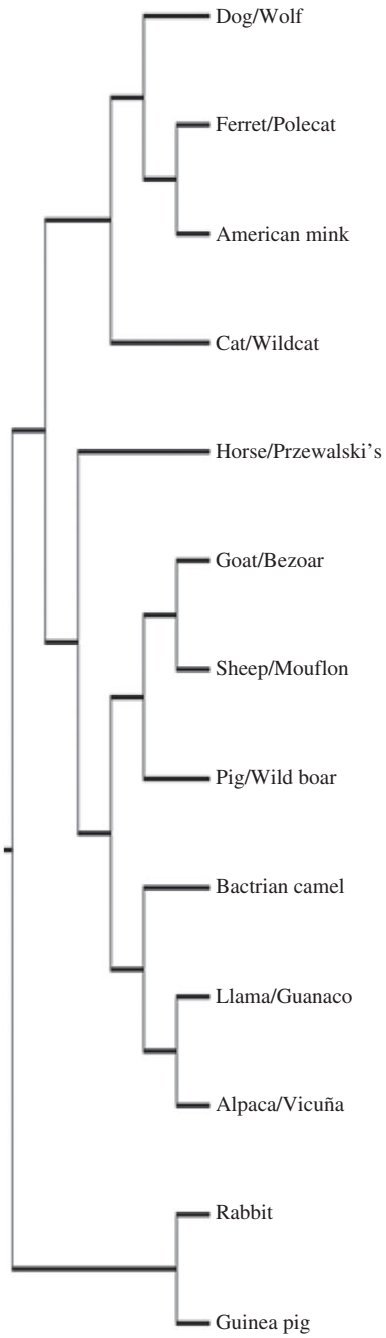
Considering all 13 comparisons wild versus domesticated (electronic supplementary material, 2), there are clear differences in the amount of change of variables, expanding one order of magnitude (table 1). The variables showing lower added change values are the breadth of the braincase, the length of the orbit and the length of the palate (0.035, 0.0460 and 0.0461, respectively). The length of the nasals is the variable showing the greatest amount of added change (0.161). The variables correlated with the trophic apparatus, upper and lower tooth rows, the height of the coronoid process and zygomatic width, have the following added change values: 0.149, 0.139, 0.130 and 0.112, respectively.

In general, the wild forms exhibit more isometric growth than the domesticated counterparts, which show more allometric growth (table 2). The number of measurements with isometric growth in wild and domesticated form is the following: wolf–dog: 2 : 1, polecat–ferret: 7 : 4, wild–domesticated mink: 7 : 6, wild–domesticated cat: 6 : 4, Przewalski's horse–horse: 7 : 4, bezoar–goat: 8 : 1, mouflon–sheep: 5 : 2, guanaco–llama: 4 : 3, vicuña–alpaca: 9 : 2, wild–domesticated rabbit: 1 : 0. In only three cases of 13 is there more isometric growth in the domesticated form, with the following numbers: boar–pig: 0 : 3; wild–domesticated Bactrian camel: 4 : 8; wild–domesticated guinea pig: 7 : 9 (table 2).

Based on the comparison of growth trajectories between domesticated and wild forms (figure 3), we found that the total number of heterochronic events was high (14) in dog–wolf, cat, horse–Przewalski's

Table 2. Summary of allometric trends in the 13 wild and domesticated forms for 14 skull variables investigated. The used symbols are: ''+'' (accelerated with respect to overall size or positive allometric), ''−'' (decelerated with respect to overall size or negative allometric), ''='' (isometric). U, untrimmed sample of pseudovalues; T, trimmed sample of pseudovalues. Abbreviations as in figure 2.

forms	minor bias	CPL	LN	HM	URP	LP	BP	LO	ZB	BB	HO	LD	HD	HC	LPR	totals
Canis lupus familiaris	U	+	+	−	+	=	−	−	−	−	−	+	−	+	+	6P,1I,7N
Canis lupus lupus	T	−	+	+	=	−	−	−	+	−	+	+	=	+	−	6P,2I,6N
Mustela putorius furo	T	+	−	=	+	+	−	−	=	−	=	+	−	+	=	5P,4I,5N
Mustela putorius putorius	U	=	+	=	=	+	−	−	=	−	−	=	=	+	=	3P,7I,4N
Neovison vison	U	−	=	+	−	=	−	−	=	−	=	=	+	=	−	2P,6I,6N
Neovison vison letifera	U	−	=	=	−	=	−	−	=	−	=	=	+	+	=	2P,7I,6N
Felis silvestris catus	U	=	+	=	−	=	+	=	+	−	−	+	+	+	−	6P,4I,4N
Felis silvestris lybica	T	−	=	=	=	=	=	−	=	−	−	+	+	+	−	3P,6I,5N
Equus ferus caballus	U	+	+	+	=	+	−	−	−	−	=	+	=	+	=	6P,4I,4N
Equus ferus przewalskii	U	=	+	+	=	+	=	−	=	−	−	=	=	+	=	4P,7I,3N
Capra hircus	U	+	+	+	=	+	−	−	−	−	−	?	?	?	?	4P,1I,5N
Capra aegagrus	U	=	=	=	=	=	=	−	=	−	=	?	?	?	?	8I,2N
Ovis aries	U	+	+	+	=	+	−	−	−	−	−	=	−	+	+	6P,2I,6N
Ovis musimon	U	=	+	+	=	=	=	−	−	−	−	=	−	+	+	4P,5I,5N
Sus scrofa domestica	U	=	+	−	+	+	−	−	−	−	−	=	=	+	+	5P,3I,6N
Sus scrofa scrofa	U	+	+	−	+	+	−	−	−	−	−	+	+	+	+	8P,0I,6N
Lama glama	U	+	+	=	=	+	−	−	−	−	=	+	−	+	−	5P,3I,6N
Lama guanicoe	T	−	+	=	−	+	=	−	−	−	−	−	=	+	=	3P,4I,7N
Lama pacos	T	−	+	=	−	=	−	−	−	−	−	−	−	−	−	1P,2I,11N
Vicugna vicugna	U	=	+	=	=	=	−	−	=	−	=	=	−	=	=	1P,9I,4N
Camelus bactrianus	U	=	?	+	=	+	−	−	=	−	=	=	=	=	=	2P,8I,3N
Camelus ferus	T	=	?	=	=	+	−	−	+	−	−	=	−	+	+	4P,4I,5N
Oryctolagus c. f. domesticus	T	+	+	+	−	+	−	−	−	−	−	+	+	+	−	7P,7N
Oryctolagus cuniculus	U	=	+	−	−	+	−	−	−	−	−	+	+	+	−	5P,1I,8N
Cavia porcellus	U	=	=	=	−	=	+	−	=	−	−	=	=	=	=	1P,9I,4N
Cavia aperea	U	=	+	=	=	+	=	−	=	−	−	=	−	=	−	2P,7I,5N



slope	intercept	shift	variables
3(14) acceleration	5(9) post-displacement	0(3)	D > W
3(14) deceleration	0(9)	3(3) hypomorphosis	W > D
4(14) acceleration	1(10) post-displacement	0(3)	D > W
0(14)	1(10) pre-displacement	3(3) hypomorphosis	W > D
0(14)	5(12) post-displacement	0(0)	D > W
2(14) deceleration	2(12) pre-displacement	0(0)	W > D
0(14)	4(10) post-displacement	0(5)	D > W
4(14) deceleration	1(10) pre-displacement	5(5) hypomorphosis	W > D
3(14) acceleration	1(9) post-displacement	4(4) hypermorphosis	D > W
2(14) deceleration	4(9) pre-displacement	0(4)	W > D
1(10) acceleration	1(8) post-displacement	3(3) hypermorphosis	D > W
1(10) deceleration	4(8) pre-displacement	0(3)	W > D
2(14) acceleration	2(12) post-displacement	8(8) hypermorphosis	D > W
0(14)	2(12) pre-displacement	0(8)	W > D
0(14)	6(13) post-displacement	0(0)	D > W
1(14) deceleration	4(13) pre-displacement	0(0)	W > D
0(13)	1(13) post-displacement	0(0)	D > W
0(13)	0(13)	0(0)	W > D
0(14)	3(9) post-displacement	0(5)	D > W
5(14) deceleration	1(9) pre-displacement	5(5) hypomorphosis	W > D
2(14) acceleration	4(6) post-displacement	0(0)	D > W
6(14) deceleration	1(6) pre-displacement	0(0)	W > D
2(14) acceleration	2(7) post-displacement	2(2) hypermorphosis	D > W
5(14) deceleration	3(7) pre-displacement	0(2)	W > D
1(14) acceleration	2(9) post-displacement	0(0)	D > W
4(14) deceleration	0(9)	0(0)	W > D

Figure 3. Summary of results of the bivariate analysis of ontogenetic trajectories of 14 skull variables in the investigated 13 wild and domesticated forms. The symbols indicate the cases in which domesticates (D) were larger than wild (W) forms (or vice versa) out of the 14 relations between size (with geometric mean as a proxy for it) and the variable in question. For those trajectories that exhibit the same slopes and intercepts, we evaluated which form, if any, exhibits significant extensions of the trajectory with respect to the other (shift). The terminology of heterochrony follows Reilly *et al*. [33]. See electronic supplementary information for a detailed list of the changes for the individual variables (figure 2).

horse, sheep, llama and rabbit, whereas in camel and guinea pig the number was low (1 and 7, respectively).

Most domesticated forms have growth trajectories different from their respective wild counterparts (figure 3, electronic supplementary material, 4, 5) with regard to the slopes (e.g. New World camelids, rabbits, dogs, cats, horses and guinea pigs). By contrast, in pigs and camels the slopes are mostly shared, with differences recorded in the intercepts (figure 3).

Common heterochronic patterns across comparisons between domesticated and wild regressions exist—here we refer to changes in slope and intercept, and when these are similar, to 'shifts' (figure 3). Except in the case of the mink, where there is no shift difference between both forms, in carnivorans the domestic forms show a shorter growth trajectory in contrast to the wild forms (figure 3; electronic supplementary material, 3, 4). This change we consider a pattern of hypomorphosis. By contrast, in other species, which are herbivores, we recorded an extension of the growth trajectory in most domesticated forms (horse, goat, sheep and rabbit) corresponding to hypermorphosis as the heterochronic pattern (figure 3). In others, such as pig, Bactrian camel, guinea pig and vicuña, there is no difference with the wild form in the extension of the growth trajectory. Just in the guanaco–llama case, the shift is higher in the wild form. In most comparisons between wild and domesticated forms, the neurocranial variables showed significant differences in their slopes, intercepts or shift. Our results based on multivariate and bivariate approaches are in general in agreement. Those multivariate coefficients of allometry that show differences in their confidence intervals (i.e. no overlap, table 1) also exhibit significant differences in the slope of ontogenetic regressions (figure 3; electronic supplementary material, 3, 4).

Bivariate regressions show in most cases high values of correlation of cranial variables with the geometric mean, with exceptions (electronic supplementary material, 5). In most cases, the lower correlation corresponds to the breadth of the braincase (e.g. wolf 0.0014; vicuña 0.1301; guanaco 0.1655; domesticated guinea pig 0.2401; wild cat 0.2507, polecat 0.2640, horse 0.3670; alpaca 0.3723; sheep 0.4078; mouflon 0.5567; wild mink 0.5631; bezoar 0.5839).

4. Discussion

The investigated species have different trajectory patterns, and the changes in trajectories recorded in wild versus domesticated forms are not equal across mammals. There is no single, universal pattern of heterochrony associated with domestication. The recognition of some common features across species as part of the 'domestication syndrome' [34] should not lead to the assumption of commonality in domestication. In fact, the phenotypic patterns of change reported for canids are not universal across mammals [5]. The same is true for ontogeny.

The differences across species in the amount of change in skull proportions with growth (table 1) probably constitute one of the most important factors affecting the amount of morphological diversity a species can attain through selective breeding—their evolvability [35]. Non-isometric (allometric) growth of species (table 2) has been considered as an indicator that the domestication process of that species may have more potential for generating morphological disparity [16,36]. This is because with even minor changes in size, allometric growth produces different proportions and in some cases thus disparity. In contrast, isometric growth implies that two individuals of different size basically look alike. For instance, for wolves, the large amount of allometric change—characteristic of canids [36]—suggest an intrinsic propensity for change—given its allometric growth. In the dog, as in the wolf, most variables also exhibit the allometric growth pattern (table 2). The isometric growth of cats has been claimed to explain at least in part the relative conservatism in this species when compared with dogs [16,36–38]. In our study, we found that with domestication there is more allometric growth (table 2). For example, wild cats exhibit more isometric growth than domesticated ones (table 2).

Insights are also provided by comparing across species the bivariate trajectories. These are slightly more conserved in cats than in dogs (figure 3). In cats, wild and domesticated forms share the same slopes for most variables, with some differences mainly in the intercepts (i.e. pre- and post-displacement) and shifts (i.e. trajectory extension), whereas in the case of dogs more variables showed different slopes (figure 3). Indeed, the added change between wild and domesticated dogs in multivariate analyses is higher than in all other pairs of wild versus domesticated analysed (table 1).

The boar, the wild rabbit and the wolf are in their wild forms the species with the highest proportion of allometric growth, with just 0, 1 and 2 isometric relations, respectively (table 2). The disparity in the domesticated forms of these three cases is large and much larger than in other cases of domestication

[16]—thus supporting the link between allometric growth and potential to become morphological diverse. Dogs and pigs are reportedly more morphologically diverse than cats and horses [16,39]. The skull disparity among domesticated rabbit populations that has evolved since the domestication in the Middle Ages [40,41] has not been quantified, but the external phenotype hints that this could be significant [42].

By virtue of having compared the most generalized domesticated form to each wild form of a species, we can approximate the changes having taken place in the initial phases of domestication [43], which concerns mostly the attainment of tameness and the skull changes and other features associated with it [34]. As such, we are not concerned here with the generation of the extremes of morphospace occupation that result from intense selection for a trait or the accentuation of particular features in special breeds. Thus, the amount of change we record in allometric patterns between wild and domesticated forms in our study reflect more an intrinsic aspect of the species growth as opposed to the effects of selection.

4.1. Developmental repatterning

Heterochrony or changes in developmental timing have been proposed as common or at least relevant in understanding changes in domestication [39]. This is confirmed for the skull, but the pattern is neither global nor uniform across species. Our results demonstrate different heterochronic patterns describe the differences between wild and domestic forms for different regions of the skull (figure 3). Even considering specifically those variables related with the neurocranium (i.e. orbit length, breadth of the braincase and occipital height), whose negative allometry is conservative across species, the highly frequent differences in trajectory between wild and domestic forms implies diverse heterochronic processes (i.e. differences in slope, intercepts and trajectory extension).

That different parts of the skull change differently [44] was to be expected given the modularity of the skull [45,46], which shows that if developmental repatterning occurs in the form of heterochrony [47], the resulting pattern is not global. This had been found, for example, when comparing wild versus domesticated guinea pigs [48] and wild boar versus domesticated pigs [49]. Our study has been about postnatal changes, which are the ones classically studied in comparative studies of growth. Based on previous works, we know that in order to understand how ontogeny varies in evolution, prenatal changes can also be important [10,50]. This has been shown for wild versus domestics in the wolf/dog case [51], and is likely to apply to other species as well. Geiger *et al*. [51] showed that the skull shape of adult dogs is both neomorphic and paedomorphic. Dog skulls show unique features already shortly after birth, whereas at any given age, juvenile dogs exhibit skull shapes that resemble those of younger wolves.

4.2. Skull modules

There is conservatism in many aspects of postnatal growth trajectories, i.e. universal patterns of allometric or isometric growth for some of the skull parts in the wild and domesticated forms for the different species. Based on studies of diverse mammalian species and clades, it is well established that during growth the neurocranial components of the skull, mostly related to the brain and sensory organs, scale negatively whereas the splanchnocranial components, related to the masticatory apparatus scale positively with size (e.g. [21,52–55]). Not surprisingly, this trend was found for all species studied (table 2). The measured skull variables related to the splanchnocranium exhibit in general more accumulated changes than the neurocranial ones (such as breadth of the braincase and occipital height; tables 1 and 2), with the exception of the height of the coronoid process and breadth of the palate. Height of the coronoid process shows positive allometry in almost all ontogenetic series analysed, suggesting an accelerated postnatal growth related with the insertion of masticatory muscles. On the other hand, the generalized negative allometry recorded in the breadth of the palate indicates a wide palate from early stages of postnatal development, which serves as a platform for the tongue during the complex process of suction during lactation in mammals (e.g. [26].

Recently, the subject of modularity as it relates to dog domestication has been examined [56], and recent work on pig skull growth looked at modules of the skull and their differential growth [49]. The subject of modularity as affected by domestication and its relation to diversification is in its infancy, including the ontogenetic perspective to the subject, in spite of its great relevance to understand the mechanistic bases of integration [47,57].

5. Conclusion

We demonstrate that domestication has influenced postnatal growth trajectories. However, there is no 'domestication syndrome' for ontogeny, as there is no single, universal pattern that accompanies domestication. The discovered patterns exemplify the complex nature of evolutionary changes in the skull during domestication, and show that these changes cannot be simply described as cases of either neomorphy or heterochrony [58].

The conservatism in many aspects of postnatal growth trajectories means that many of the differences among species may exist already around birth, and that the study of prenatal ontogeny of the skull is also important to understand the paths of differentiation across species [10,11]. We hypothesize that domestication has influenced prenatal growth patterns, a case of 'developmental penetrance' of evolutionary change [59].

Ethics. No special permission was needed to use the existing collections of skulls in the museums visited, other than the agreement of the curators listed in the supplementary information.

Data accessibility. See electronic supplementary material.

Authors' contributions. All authors conceived the study and the methods and measurements applied, M.R.S.-V., V.S. and D.F. wrote the paper, all authors collected data, revised and approved the manuscript.

Competing interests. We have no competing interests.

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Authors' Contributions

Chapter 1

LH, MRSV, and MC conceived and designed the study. LH carried out the data collection and analysis, as well as the manuscript drafting. All authors contributed to the manuscript modification and finalization.

Chapter 2

LH, MRSV, and MC conceived and designed the study. LH carried out the data collection. LH and MC analyzed the data. LH drafted the manuscript. All authors contributed to the manuscript modification and finalization.

Chapter 3

LH and MRSV conceived and designed the study. LH collected the data and performed and interpreted the geometric morphometric analyses. AE provided the R functions used in the geometric morphometric analyses. LABW performed and interpreted the modularity analyses. MS performed Foote's partial disparity analyses and supported LH during the geometric morphometric analyses. LH drafted the manuscript and LABW drafted the part on modularity within the manuscript. All authors contributed to the manuscript modification and finalization.

Chapter 4

LH, MS, and MRSV conceived and designed the study. LH collected the data. LH and MS performed and interpreted the analyses. LH drafted the manuscript. All authors contributed to the manuscript modification and finalization.

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RESEARCH ARTICLES

Heck, L., Clauss, M. & Sánchez-Villagra, M. R. (2017). *Do domesticated mammals selected for intensive production have less variable gestation periods?*. Mammalian Biology-Zeitschrift für Säugetierkunde

Sánchez-Villagra, M. R., Segura, V., Geiger, M., **Heck, L.**, Veitschegger, K. & Flores, D. (2017). *On the lack of a universal pattern associated with mammalian domestication - differences in skull growth trajectories across phylogeny.* Royal Society Open Science

Heck, L., Clauss, M. & Sánchez-Villagra, M. R. (2017). *Gestation length variation in domesticated horses and its relation to breed and body size diversity.* Mammalian Biology-Zeitschrift für Säugetierkunde 84 : 44-51.

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Schierwater, B., Eitel, M., Osigus, H. J., von der Chevallerie, K., Bergmann, T., Hadrys, H., Cramm, M., **Heck, L.** , Jakob, W., Lang, M. R. & DeSalle, R. (2010). *Trichoplax and Placozoa: one of the crucial keys to understanding metazoan evolution*. Key transitions in animal evolution, 289-326.

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01.2015 -12.2017	PhD representative in the Academic Committee and the Biology Division Council, MNF, University of Zurich
12.2015	SYNTHESYS Scholarship to visit the mammal collection at the NHM Vienna

POSTER PRESENTATIONS AND TALKS

09.2017	Talk, Heck L., Stange M., Clauss M. and Sánchez-Villagra, M. R., *Morphological disparity and developmental shape changes in domesticated horses,* 110. Jahrestagung der Deutschen Zoologischen Gesellschaft, Bielefeld, Germany
08.2017	Talk, Heck L., Stange M., Clauss M. and Sánchez-Villagra, M. R., *On horse domestication: skull morphospace and life history changes.* BioSystEU 2017, Gothenburg, Sweden
07.2016	Abstract, Heck L. and Sánchez-Villagra, M. R., *Morphological disparity, growth and life history variation in domesticated horses,* ICVM, Washington, USA
05.2016	Poster, Heck L. and Sánchez-Villagra, M. R., T*he Falabella – Morphologically special or just a small horse?*, 30. JAPV, Buenos Aires, Argentina
09.2015	Talk, Heck L., Clauss M. and Sánchez-Villagra, M. R., *Gestation length and life history diversity among horse breeds*, DGS Meeting 2015, Hannover, Germany

OUTREACH

01.2017	Talk, *Vom Beutetier zum Gefährten: Wie veränderte die Domestizierung das Pferd?* at the Palaeontological Institute and Museum, University of Zurich
12.2015	Organisation of the Science Trail at the Palaeontological Institute and Museum, University of Zurich
09.2015	Talk, *Die Domestizierung des Pferdes - Vom Beutetier zum Gefährten,* Lange Nacht der Zürcher Museen, Palaeontological Institute and Museum, University of Zurich